US03/03957

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/54, 9/12, C07K 16/40, A61K 38/45, C12Q 1/48, C12N 15/11 — A2

(11) International Publication Number: WO 98/11234

(43) International Publication Date: 19 March 1998 (19.03.98)

(21) International Application Number: PCT/US97/15923

(22) International Filing Date: 10 September 1997 (10.09.97)

(30) Priority Data:
08/712,709 12 September 1996 (12.09.96) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/712,702 (CIP)
Filed on 12 September 1996 (12.09.96)

(71) Applicant *(for all designated States except US)*: INCYTE PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive, Palo Alto, CA 94304 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: AU-YOUNG, Janice [US/US]; 1419 Kains Avenue, Mountain View, CA 94702 (US). GUEGLER, Karl, J. [CH/US]; 1048 Oakland Avenue, Menlo Park, CA 94025 (US). HAWKINS, Phillip, R. [US/US]; 750 N. Shoreline Boulevard #96, Mountain View, CA 94034 (US).

(74) Agent: BILLINGS, Lucy, J.; Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304 (US).

(81) Designated States: AT, AU, BR, CA, CH, CN, DE, DK, ES, FI, GB, IL, JP, KR, MX, NO, NZ, RU, SE, US, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: HUMAN PROTEIN KINASES

```
                9          18         27         36         45         54
5' NNC ATT CTG GGA CCT GTT CGC AGG ACC GTC CGG TGT TCT GGC CCC CTG ATG TCA

               63         72         81         90         99        108
CCT TCA CGG GCC TGA CTC ACA GTC CTA AAT ATC TGA CAG CGA AGA TCG CTT GTA

              117        126        135        144        153        162
GTT CGT GCC CTC GTG AGG CTG GCA TGC AGG ATG GCA GGA CAG CCC GGC CAC ATG

              171        180        189        198        207        216
CCC CAT GGA GGG AGT TCC AAC AAC CTC TGC CAC ACC CTG GGG CCT GTG CAT CCT

              225        234        243        252        261        270
CCT GAC CCA CAG AGG CAT CCC AAC ACG CTG TCT TTT CGC TGC TCG CTG GCG GAC

              279        288        297        306        315        324
TTC CAG ATC GAA AAG AAG ATA GGC CGA GGA CAG TTC AGC GAG GTG TAC AAG GCC

              333        342        351        360        369        378
ACC TGC CTG CTG GAC AGG AAG ACA GTG GCT CTG RAG AAG GTG CAG ATC TTT GAG

              387        396        405        414        423        432
ATG ATG GAC GCC AAG GCG AAG CAG GAC TGT GTC AAG GAG ATC GGC CTC TTG AAG
M   M   D   A   K   A   K   Q   D   C   V   K   E   I   G   L   L   K
```

(57) Abstract

The present invention provides novel human protein kinases (HPK) and polynucleotides which identify and encode HPK. The invention provides for genetically engineered expression vectors and host cells comprising the nucleic acid sequences encoding HPK. The invention also provides for pharmaceutical compositions comprising HPK or antagonists of HPK, and antibodies which specifically bind HPK. Additionally, the invention provides antisense molecules to HPK for treatment or prevention of diseases associated with abnormal expression of HPK.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

HUMAN PROTEIN KINASES

TECHNICAL FIELD

The present invention relates to nucleic acid and amino acid sequences of novel human protein kinases and to the use of these sequences in the diagnosis, study, prevention and treatment of disease.

BACKGROUND ART

Kinases regulate many different cell proliferation, differentiation, and signalling processes by adding phosphate groups to proteins. Uncontrolled signalling has been implicated in a variety of disease conditions including, inflammation, cancer, arteriosclerosis, and psoriasis. Reversible protein phosphorylation is the main strategy for controlling activities of eukaryotic cells. It is estimated that more than 1000 of the 10.000 proteins active in a typical mammalian cell are phosphorylated. The high energy phosphate which drives activation is generally transferred from adenosine triphosphate molecules (ATP) to a particular protein by protein kinases and removed from that protein by protein phosphatases.

Phosphorylation occurs in response to extracellular signals (hormones, neurotransmitters, growth and differentiation factors, etc), cell cycle checkpoints, and environmental or nutritional stresses and is roughly analogous to turning on a molecular switch. When the switch goes on, the appropriate protein kinase activates a metabolic enzyme, regulatory protein, receptor, cytoskeletal protein, ion channel or pump, or transcription factor.

The kinases comprise the largest known protein group, a superfamily of enzymes with widely varied functions and specificities. They are usually named after their substrate, their regulatory molecules, or some aspect of a mutant phenotype. Almost all kinases contain a similar 250-300 amino acid catalytic domain. The N-terminal domain, which contains subdomains I-IV, generally folds into a two-lobed structure which binds and orients the ATP (or GTP) donor molecule. The larger C terminal lobe, which contains subdomains VI A-XI, binds the protein substrate and carries out the transfer of the gamma phosphate from ATP to the hydroxyl group of a serine, threonine, or tyrosine residue. Subdomain V spans the two lobes.

The kinases may be categorized into families by the different amino acid sequences (generally between 5 and 100 residues) located on either side of, or inserted into loops of, the kinase domain. These added amino acid sequences allow the regulation of each kinase as it recognizes and interacts with its target protein. The primary structure of the kinase domains is conserved and can be further subdivided into 11 subdomains. Each of the 11 subdomains contain

specific residues and motifs or patterns of amino acids that are characteristic of that subdomain and are highly conserved (Hardie G and Hanks S (1995) The Protein Kinase Facts Books, I and II, Academic Press, San Diego CA).

The second messenger dependent protein kinases primarily mediate the effects of second messengers such as cyclic AMP (cAMP) cyclic GMP, inositol triphosphate, phosphatidylinositol, 3,4,5-triphosphate, cyclic ADPribose, arachidonic acid and diacylglycerol. Cyclic-AMP is an intracellular mediator of hormone action in all procaryotic and animal cells that have been studied. Such hormone-induced cellular responses include thyroid hormone secretion, cortisol secretion, progesterone secretion, glycogen breakdown, bone resorption, and regulation of heart rate and force of heart muscle contraction. Cyclic AMP-dependent protein kinase (PKA) is found in all animal cells and is thought to account for the all of the effects of cyclic-AMP in most of these cells. In its inactive state, A-kinase consists of a complex of two catalytic subunits and two regulatory subunits. When each regulatory subunit has bound two molecules of cAMP, the catalytic subunit is activated and can transfer a high energy phosphate from ATP to the serine or threonine of a substrate protein. Altered PKA expression is implicated in a variety of disorders and diseases including; thyroid disorders, diabetes, atherosclerosis, and cardiovascular disease (Isselbacher KJ et al (1994) Harrison's Principles of Internal Medicine, McGraw-Hill, New York City).

Protein kinase C (PKC) is a water-soluble, Ca^{++}-dependent kinase, commonly found in brain tissue, which moves to the plasma membrane in the presence of Ca^{++} ions. Approximately half of the known isoforms of PKC are activated initially by diacylglycerol and phosphatidylserine. Prolonged activation of PKC depends on continued production of diacyglycerol molecules which are formed when phospholipases cleave phosphatidylcholine. In nerve cells, PKC phosphorylates ion channels and alters the excitability of the cell membrane. In other cells, activation of PKC increases gene transcription either by triggering a protein kinase cascade which activates a regulatory element or by phosphorylating and deactivating an inhibitor of the regulatory protein. PKC activity has been specifically linked to multi-drug resistance in cancer (O'Brian CA et al (1995) Prog Clin Biol Res 391: 117-120), tumor promotion (O'Brian CA and Ward NE (1989) Cancer Metast Rev 8: 199-214) memory disorders (Saito N. et al (1994) Brain Res 656: 245-256), and auto-immune disease (Ohkusu K et al (1995) Eur J Immunol 25: 3180-3186).

A detailed understanding of kinase pathways and signal transduction is beginning to

reveal some mechanisms for interceding in the progression of inflammatory illnesses and of uncontrolled cell proliferation. The novel kinases,polynucleotides which encode them, and antibodies to them satisfy a need in the art by providing a plurality of tools for studying signalling cascades in various cells and tissues, diagnosing disease and selecting inhibitors or drugs with the potential to intervene in various disorders or diseases in which altered kinase expression is implicated.

DISCLOSURE OF THE INVENTION

The present invention is directed to three novel human protein kinases (hereinafter referred to individually as HPK1, HPK2, and HPK3, and collectively as HPK) characterized as having homology to other protein kinases. Accordingly, the invention features substantially purified HPK, comprising the amino acid sequences of SEQ ID NOs:1, 3, and 5,or fragments thereof and having functional characteristics of protein kinase family members.

One aspect of the invention features isolated polynucleotides which encode all or a part of HPK. In a particular aspect, the polynucleotides are the nucleotide sequences shown in SEQ ID NOs:2, 4, and 6. Also provided are vectors containing such polynucleotides and host cells transformed or transfected with such vectors.

The invention further relates to poylynucleotide sequences complementary to the polynucleotides encoding HPK or variants thereof, antibodies or antagonists to HPK, and pharmaceutical compositions comprising HPK or antagonists to HPK.

BRIEF DESCRIPTION OF DRAWINGS

Figures 1A, 1B, 1C and 1D show the nucleic acid sequence (SEQ ID NO:2) and amino acid sequence (SEQ ID NO:1) of the human protein kinase, HPK-1. The alignment was produced using MacDNAsis software (Hitachi Software Engineering Co Ltd, San Bruno, CA).

Figures 2A, 2B, 2C, 2D, 2E and 2F show the nucleic acid sequence (SEQ ID NO:4) and amino acid sequence (SEQ ID NO:3) of the human protein kinase, HPK-2.

Figures 3A, 3B, 3C, 3D, 3E and 3F show the nucleic acid sequence (SEQ ID NO:6) and amino acid sequence (SEQ ID NO:5) of the human protein kinase, HPK-3.

Figures 4A, 4B, 4Cand 4D show the amino acid sequence alignments between HPK-1, HPK-2, HPK-3 and protein kinases from the nematode, C. elegans (GI 1082115; SEQ ID NO: 7), a human protein kinase (GI 1117791; SEQ ID NO: 8), and a protein kinase from rat (GI 294637; SEQ ID NO: 9). The alignments were produced using the multisequence alignment program of DNAStar software (DNAStar Inc, Madison WI).

MODES FOR CARRYING OUT THE INVENTION

Before the present nucleotide and polypeptide sequences are described, it is to be understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors and reagents described as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein an in the appended claims, the singular forms of "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, referrence to "a host cell" includes a plurality of such host cells and referrence to "the antibody" includes reference to one or more antibodies and equivalents thereof known to those skilled in the arts, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs. Although any methods, devices and materials similar or equivalent to those described herein can be used in the practice of testing of the invention, the preferred methods, devices and materials are now described.

All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are described in the publications which might be used in connection with the presently described invention. The publications discussed herein are provided solely for their disclosure pprior sto the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

Definitions

"Nucleic acid sequence" as used herein refers to an oligonucleotide, nucleotide or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represent the sense or antisense strand. Similarly, amino acid sequence as used herein refers to protein or peptide sequence.

"Consensus" as used herein may refer to a nucleic sequence 1) which has been resequenced to resolve uncalled bases, 2) which has been extended using XL-PCR (Perkin Elmer) in the 5' or the 3' direction and resequenced, 3) which has been assembled from overlapping sequences of more than one Incyte clone GCG Fragment Assembly System, (GCG,

Madison WI), or 4) which has been both extended and assembled.

"Peptide nucleic acid" as used herein refers to a molecule which comprises an oligomer to which an amino acid residue, such as lysine, and an amino group have been added. These small molecules, also designated anti-gene agents, stop transcript elongation by binding to their complementary (template) strand of nucleic acid (Nielsen PE et al (1993) Anticancer Drug Des 8:53-63).

As used herein, HPK refers to the amino acid sequence of substantially purified HPK from any source whether natural, synthetic, semi-synthetic or recombinant.

A "variant" of HPK is defined as an amino acid sequence that is different by one or more amino acid substitutions. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, eg, replacement of leucine with isoleucine. More rarely, a variant may have "nonconservative" changes, eg, replacement of a glycine with a tryptophan. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which and how many amino acid residues may be substituted, inserted or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNAStar software.

A "deletion" is defined as a change in either nucleotide or amino acid sequence in which one or more nucleotides or amino acid residues, respectively, are absent.

An "insertion" or "addition" is that change in a nucleotide or amino acid sequence which has resulted in the addition of one or more nucleotides or amino acid residues, respectively, as compared to the naturally occurring HPK.

A "substitution" results from the replacement of one or more nucleotides or amino acids by different nucleotides or amino acids, respectively.

The term "biologically active" refers to a HPK having structural, regulatory or biochemical functions of the naturally occurring HPK. Likewise, "immunologically active" defines the capability of the natural, recombinant or synthetic HPK, or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The term "derivative" as used herein refers to the chemical modification of a nucleic acid sequence encoding HPK or the encoded HPK. Illustrative of such modifications would be replacement of hydrogen by an alkyl, acyl, or amino group. A nucleic acid derivative would encode a polypeptide which retains essential biological characteristics of natural HPK.

As used herein, the term "substantially purified" refers to molecules, either nucleic or amino acid sequences, that are removed from their natural environment, isolated or separated, and are at least 60% free, preferably 75% free, and most preferably 90% free from other components with which they are naturally associated.

"Stringency" typically occurs in a range from about Tm-5°C (5°C below the Tm of the probe)to about 20°C to 25°C below Tm. As will be understood by those of skill in the art, a stringency hybridization can be used to identify or detect identical polynucleotide sequences or to identify or detect similar or related polynucleotide sequences.

The term "hybridization" as used herein shall include "any process by which a strand of nucleic acid joins with a complementary strand through base pairing" (Coombs J (1994) Dictionary of Biotechnology, Stockton Press, New York NY). Amplification as carried out in the polymerase chain reaction technologies is described in Dieffenbach CW and GS Dveksler (1995, PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview NY).

Description

The present invention relates to novel human protein kinases, HPK, initially identified among the partial cDNAs from a brain hippocampus library (HIPONOT01; HPK-1), a peripheral blood mononuclear cell library (TMLR3DT01; HPK-2) and a macrophage cell library (MPHGN0T03; HPK-3) and to the use of the nucleic acid and amino acid sequences disclosed herein in the study, diagnosis, prevention and treatment of disease.

In addition to the above mentioned sources, northern analysis indicates that nucleic acid encoding a portion of HPK-1 was also found in cDNA libraries from neural tissue (multiple sclerosis) and brain tumor. Nucleic acid encoding portions of HPK-2 was found in infant brain, epilepsy (brain) and various tumor tissues (penis carcinoma, bladder carcinoma, and thyroid adenoma). Nucleic acid encoding portions of HPK-3 was found in multiple sclerosis, Alzheimers (brain), osteoarthritic knee tissue, and in tumors of the breast and lung.

The present invention also encompasses HPK variants. A preferred HPK variant is one having at least 80% amino acid sequence similarity to the HPK amino acid sequences (SEQ ID NO:1, 3, or 5), a more preferred HPK variant is one having at least 90% amino acid sequence similarity to SEQ ID NO:1, 3, or 5, and a most preferred HPK variant is one having at least 95% amino acid sequence similarity to SEQ ID NO:1, 3, or 5.

The HPK Coding Sequences

Nucleic acid encoding a portion of HPK-1 was first identified in the cDNA, Incyte Clone

240142, through a computer-generated search for amino acid sequence alignments. Similarly, nucleic acids encoding a portion of HPK-2 and HPK-3 were first identified in Incyte Clones 391602 and 477245, respectively. The nucleic acid sequences, SEQ ID NO:2, 4, and 6; disclosed herein encode the amino acid sequences, SEQ ID NO:1, 3, and 5, respectively, disclosed hereinafter as HPK.

The present invention is based, in part, on the chemical and structural homology among HPK-1, -2, and -3, and various known protein kinases, and to various amino acid sequence motifs within these proteins that are characteristic of the catalytic domains of protein kinases (Hardie G and Hanks S (1995), supra). Referring to Figures 4A, 4B, and 4C, the sequence GXGXXGXV characteristic of subdomain I in protein kinases is found in HPK-2 beginning at G_{37} and in the corresponding residues for HPK-3, GI 1117791, and GI 294637. The conserved lysine residue in subdomain II located at K_{49} for HPK-2 is repeated for HPK-3, GI 1117791, and GI 294637. The sequence HRDIKXXN found in subdomain VI B of many protein kinases is found in HPK-1(H_{90}), HPK-2, HPK-3, GI 1082115 and GI 1117791. Finally, the triplet sequence DFG in subdomain VII is found in HPK-3 (G_{242}), GI 1117791, and GI 294637, and the triplet sequence APE (subdomain VIII) is found in HPK-2 (A_{283}), HPK-3, GI 1117791, and GI 294637.

Thus each of the protein kinases HPK-1, -2, and -3 bear sequence patterns characteristic of protein kinases, but are distinct from one another in overall sequence. HPK-1 bears 70% sequence identity to a protein kinase from the nematode, C. elegans: GI 1082115 (Wilson, R et al (1994) Nature 368: 32-38). GI 1082115 has been characterized as a member of the cyclic-AMP dependent PKA family. HPK-2 bears closest identity (42%)to a human protein kinase: GI 1117791 (Creasy, CL and Chernoff, J (1995) J. Biol Chem 270: 21695-21700). GI 1117791 is characterized as being similar to other members of the mitogen-activated protein kinase (MAPK) family but is most likely involved in an as yet unidentified signal transduction pathway. HPK-3 has approximately 96% identity to a protein kinase from rat; GI 294637 (Webster, M.K. et al (1993) Mol. Cell Biol. 13: 2031-2040). GI 294637 is transcriptionally regulated by glucocorticoid hormones and bears sequence homology to protein kinases of both the PKA and PKC families.

HPK-1 is encoded by SEQ ID NO:2 and is derived from the extension and assembly of the following partial cDNAs(library), Incyte Clones 67192(HUVESTB01); 240142, 243638, and 298165(HIPONOT01); 449634(TLYMNOT02); 461400(KERANOT01); 739131(PANCNOT04); and (12143028?).

HPK-2 is encoded by SEQ ID NO:4 and is derived from the extension and assembly of the following partial cDNAs, Incyte Clones 1394374, 1395924, 1392440, 1394764, 1393587, and 1439946(THYRNOT03; 487890(HNT2AGT01); 737620(TONSNOT01); 391602(TMLR3DT01); 373301(LUNGNOT02); 1291632(PGANNOT03); 550890(BEPINOT01); 1314539(BLADTUT02); 647351(BRSTTUT02); 917302(BRSTNOT04), 541117(LNODNOT02); 235796(SINTNOT02); 827973(PROSNOT06); 36252(HUVENOB01); 1339623(COLNTUT03); 719820 and 365833(SYNORAT01); 32632(THP1NOB01); 888061(PANCNOT05); 1262882(SYNORAT05); 975808(MUSCNOT02); 275375(TESTNOT03);1433039 and 1425069(BEPINON01);and 94156(PITUNOT01).

HPK-3 is encoded by SEQ ID NO:6 and is derived from the extension and assembly of the following partial cDNAs, Incyte Clones 477245 and 445652(MPHGNOT03); 386314(THYMNOT02); 1219404(NEUTGMT01); 478857(MMLR2DT01); 1239468(LUNGTUT02); 603976(BRSTTUT01; and 565613(NEUTLPT01).

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of HPK-encoding nucleotide sequences, some bearing minimal homology to the nucleotide sequences of any known and naturally occurring gene may be produced. The invention contemplates each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of naturally occurring HPK, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode HPK and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring HPK under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding HPK or its derivatives possessing a substantially different codon usage. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic expression host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HPK and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

It is now possible to produce a DNA sequence, or portions thereof, encoding any of the

claimed HPK and derivatives, entirely by synthetic chemistry, after which the synthetic gene may be inserted into any of the many available DNA vectors and cell systems using reagents that are well known in the art at the time of the filing of this application. Moreover, synthetic chemistry may be used to introduce mutations into a HPK sequence or any portion thereof.

Also included within the scope of the present invention are polynucleotide sequences that are capable of hybridizing to the nucleotide sequence of Figures 1A, 1B, 1C and 1D under various conditions of stringency. Hybridization conditions are based on the melting temperature (Tm) of the nucleic acid binding complex or probe, as taught in Berger and Kimmel (1987, Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol 152, Academic Press, San Diego CA) incorporated herein by reference, and may be used at a defined stringency.

Altered nucleic acid sequences encoding HPK which may be used in accordance with the invention include deletions, insertions or substitutions of different nucleotides resulting in a polynucleotide that encodes the same or a functionally equivalent HPK. The protein may also show deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent HPK. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the biological activity of HPK is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine phenylalanine, and tyrosine.

Included within the scope of the present invention are alleles of HPK encoding sequences. As used herein, an "allele" or "allelic sequence" is an alternative form of an HPK encoding sequence. Alleles result from a mutation, for example, a change in the nucleic acid sequence, and generally produce altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have, one or many allelic forms, or none at all. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions or substitutions of amino acids. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

Methods for DNA sequencing which are well known in the art may be used and these methods may employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase® (US Biochemical Corp, Cleveland OH)), Taq polymerase (Perkin Elmer, Norwalk CT),

thermostable T7 polymerase (Amersham, Chicago IL), or combinations of recombinant polymerases and proofreading exonucleases such as the ELONGASE Amplification System marketed by Gibco BRL (Gaithersburg MD). Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown MA) and the ABI 377 DNA sequencers (Perkin Elmer).

Extending the Polynucleotide Sequence

The polynucleotide sequence encoding HPK may be extended utilizing partial nucleotide sequence and various methods known in the art to detect upstream sequences such as promoters and regulatory elements. For example, one may use "restriction-site" polymerase chain reaction (PCR) as a direct method which uses universal primers to retrieve an unknown sequence adjacent to a known locus (Gobinda et al (1993) PCR Methods Applic 2:318-22). In particular, the genomic DNA is amplified in the presence of primer to a linker sequence and a primer specific to the known region. The amplified sequences are subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR can be used to amplify or extend sequences using divergent primers based on a known region (Triglia T et al (1988) Nucleic Acids Res 16:8186). The primers may be designed using OLIGO® 4.06 Primer Analysis Software (1992; National Biosciences Inc, Plymouth MN), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which may be used is capture PCR (Lagerstrom M et al (1991) PCR Methods Applic 1:111-19) which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA. Capture PCR involves multiple restriction enzyme digestions and ligations to place an engineered double-stranded sequence into an unknown portion of the DNA molecule before PCR.

Another method which may be used to retrieve unknown sequences is that of (Parker JD et al (1991; Nucleic Acids Res 19:3055-60). Additionally, one can use PCR, nested primers and PromoterFinder libraries to walk in genomic DNA (PromoterFinder™ Clontech, Palo Alto CA).

This process avoids the need to screen libraries and is useful in finding intron/exon junctions. Preferred libraries for screening for full length cDNAs are those that have been size-selected to include larger cDNAs. Also, random primed libraries are preferred in that they will contain more sequences which contain the 5' and upstream regions of genes. A randomly primed library may be particularly useful if an oligo d(T) library does not yield a full-length cDNA. Genomic libraries are useful for extension into the 5' nontranslated regulatory region.

Capillary electrophoresis may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. Systems for rapid sequencing are available from Perkin Elmer, Beckman Instruments (Fullerton CA), and other companies. Capillary sequencing may employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled devise camera. Output/light intensity is converted to an electrical signal using appropriate software (eg. Genotyper™ and Sequence Navigator™ from Perkin Elmer) and the entire process from loading of samples to computer analysis and electronic data display is computer controlled. Capillary electrophoresis is particularly suited to the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample. The reproducible sequencing of up to 350 bp of M13 phage DNA in 30 min has been reported (Ruiz-Martinez MC et al (1993) Anal Chem 65:2851-8).

Expression of the Nucleotide and Protein Sequences

In accordance with the present invention, polynucleotide sequences which encode HPK, fragments of the polypeptide, fusion proteins or functional equivalents thereof may be used in recombinant DNA molecules that direct the expression of HPK in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence, may be used to clone and express HPK. As will be understood by those of skill in the art, it may be advantageous to produce HPK-encoding nucleotide sequences possessing non-naturally occurring codons. Codons preferred by a particular prokaryotic or eukaryotic host can be selected, for example, to increase the rate of HPK encoding sequences expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, than transcripts produced from naturally occurring sequence (Murray E et al (1989) Nuc Acids Res 17:477-508).

The nucleotide sequences of the present invention can be engineered in order to alter HPK encoding sequences for a variety of reasons, including but not limited to, alterations which

modify the cloning, processing and/or expression of the gene product. For example, mutations may be introduced using techniques which are well known in the art. For example, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

In another embodiment of the invention, a natural, modified or recombinant sequence encoding HPK may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for inhibitors of HPK activity, it may be useful to encode a chimeric HPK protein that is recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between a HPK sequence and the heterologous protein sequence, so that the HPK may be cleaved and substantially purified away from the heterologous moiety.

In an alternate embodiment of the invention, the sequence encoding HPK may be synthesized, whole or in part, using chemical methods well known in the art (see Caruthers MH et al (1980) Nuc Acids Res Symp Ser 215-23. Horn T et al (1980) Nuc Acids Res Symp Ser 225-32, etc). Alternatively, the proteins may be produced using chemical methods to synthesize amino acid sequences, whole or in part. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge JY et al (1995) Science 269:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer) in accordance with the instructions provided by the manufacturer.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (eg, Creighton (1983) Proteins, Structures and Molecular Principles, WH Freeman and Co, New York NY). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (eg, the Edman degradation procedure; Creighton, supra). Additionally the amino acid sequence of HPK, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

Expression Systems

In order to express a biologically active HPK, the nucleotide sequence encoding HPK or its functional equivalent, is inserted into an appropriate expression vector, ie, a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence.

Methods which are well known to those skilled in the art can be used to construct

expression vectors containing a HPK coding sequence and appropriate transcriptional or translational controls. These methods include in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination or genetic recombination. Such techniques are described in Sambrook et al (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY and Ausubel FM et al (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY.

A variety of expression vector/host systems may be utilized to contain and express a HPK coding sequence. These include but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (eg, baculovirus); plant cell systems transfected with virus expression vectors (eg, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (eg, Ti or pBR322 plasmid); or animal cell systems.

The "control elements" or "regulatory sequences" of these systems may vary in their strength and specificities and are those nontranslated regions of the vector, enhancers, promoters, and 3' untranslated regions, which interact with host cellular proteins to carry out transcription and translation. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the Bluescript® phagemid (Stratagene, LaJolla CA) or pSport1 (Gibco BRL) and ptrp-lac hybrids and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (eg, heat shock, RUBISCO; and storage protein genes) or from plant viruses (eg, viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from the mammalian genes or from mammalian viruses are most appropriate. If it is necessary to generate a cell line that contains multiple copies of HPK encoding sequences, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for HPK. For example, when large quantities of HPK are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be desirable. Such vectors include, but are not limited to, the multifunctional E. coli cloning and expression vectors such as Bluescript® (Stratagene), in which the HPK encoding

sequences may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of ß-galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke & Schuster (1989) J Biol Chem 264:5503-5509); and the like. pGEX vectors (Promega, Madison WI) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to include heparin, thrombin or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, Saccharomyces cerevisiae, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase and PGH may be used. General methodology may be found in Ausubel et al (supra) and Grant et al (1987) Methods in Enzymology 153:516-544.

In cases where plant expression vectors are used, the expression of a sequence encoding HPK may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV (Brisson et al (1984) Nature 310:511-514) may be used alone or in combination with the omega leader sequence from TMV (Takamatsu et al (1987) EMBO J 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi et al (1984) EMBO J 3:1671-1680; Broglie et al (1984) Science 224:838-843); or heat shock promoters (Winter J and Sinibaldi RM (1991) Results Probl Cell Differ 17:85-105) may be used. These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. For reviews of such techniques, see Hobbs S or Murry LE in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill New York NY, pp 191-196 or Weissbach and Weissbach (1988) Methods for Plant Molecular Biology, Academic Press, New York NY, pp 421-463.

An alternative expression system which could be used to express HPK encoding sequences is an insect system. In one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The HPK encoding sequences may be cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of HPK encoding sequences will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein coat. The recombinant viruses may then be used

to infect S. frugiperda cells or Trichoplusia larvae in which HPK is expressed (Smith et al (1983) J Virol 46:584; Engelhard EK et al (1994) Proc Nat Acad Sci 91:3224-7).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, an HPK encoding sequence may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a nonessential E1 or E3 region of the viral genome will result in a viable virus capable of expressing HPK in infected host cells (Logan and Shenk (1984) Proc Natl Acad Sci 81:3655-59). In addition, transcription enhancers, such as the rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be required for efficient translation of an HPK encoding sequence. These signals include the ATG initiation codon and adjacent sequences. In cases where an HPK encoding sequence, its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon must be in the correct reading frame to ensure transcription of the entire insert. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (Scharf D et al (1994) Results Probl Cell Differ 20:125-62; Bittner et al (1987) Methods in Enzymol 153:516-544).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, 293, WI38, etc have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express HPK encoding sequences may be transformed using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells

may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes which can be employed in tk- or aprt- cells, respectively (Wigler M et al (1977) Cell 11:223-32; Lowy I et al (1980) Cell 22:817-23). Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate; npt, which confers resistance to the aminoglycosides neomycin and G-418 and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Wigler M et al (1980) Proc Natl Acad Sci 77:3567-70; Colbere-Garapin F et al (1981) J Mol Biol 150:1-14; Murry, supra). Additional selectable genes may be used, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman SC and RC Mulligan (1988) Proc Natl Acad Sci 85:8047-51). Visible markers such as anthocyanins, ß glucuronidase and its substrate, GUS, and luciferase and its substrate, luciferin, may be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes CA et al (1995) Methods Mol Biol 55:121-131).

Identification of Transformants Containing the Polynucleotide Sequence

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression should be confirmed. For example, if the HPK encoding sequence is inserted within a marker gene sequence, recombinant cells containing HPK encoding sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with an HPK sequence under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem HPK encoding sequence as well.

Alternatively, host cells which contain the HPK encoding sequence and express HPK may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridization and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the

detection and/or quantification of the nucleic acid or protein.

The presence of the polynucleotide sequence encoding HPK can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes, portions or fragments of HPK encoding sequences. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequence encoding HPK to detect transformants containing HPK encoding sequences in DNA or RNA. As used herein "oligonucleotides" or "oligomers" refer to a nucleic acid sequence of at least about 10 nucleotides and as many as about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20-25 nucleotides which can be used as a probe or amplimer.

A variety of protocols for detecting and measuring the expression of HPK, using either polyclonal or monoclonal antibodies specific for the protein are well known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HPK is preferred, but a competitive binding assay may be employed. These and other assays are described, among other places, in Hampton R et al (1990, Serological Methods, a Laboratory Manual, APS Press, St Paul MN) and Maddox DE et al (1983, J Exp Med 158:1211).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to HPK encoding sequences include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the HPK encoding sequence, or any portion of it, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labeled nucleotides. A number of commereccial kits or protocols for these procedures may be obtained from companies such as Pharmacia Biotech (Piscataway NJ), Promega (Madison WI), and US Biochemical Corp (Cleveland OH. Suitable reporter molecules or labels include those radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles and the like. Protocols for using these labels are widely available in the art. One may also produce recombinant immunoglobulins by methods provided in the art.

Purification of HPK

Host cells transformed with a nucleotide sequence encoding HPK may be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides encoding HPK can be designed with signal sequences which direct secretion of HPK through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join HPK encoding sequences to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins as described in (Kroll DJ et al (1993) DNA Cell Biol 12:441-53).

HPK may also be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle WA). The inclusion of a cleavable linker sequences such as Factor XA or enterokinase (Invitrogen, San Diego CA) between the purification domain and HPK is useful to facilitate purification. One such expression vector which provides for expression of a fusion protein comprising an HPK contains nucleic acid encoding 6 histidine residues followed by thioredoxin and an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromotography as described in Porath et al (1992) Protein Expression and Purification 3: 263-281) while the enterokinase cleavage site provides a means for purifying the neuronatin from the fusion protein.

In addition to recombinant production, fragments of HPK may be produced by direct peptide synthesis using solid-phase techniques (cf Stewart et al (1969) Solid-Phase Peptide Synthesis, WH Freeman Co, San Francisco; Merrifield J (1963) J Am Chem Soc 85:2149-2154). In vitro protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster City CA) in accordance with the instructions provided by the manufacturer. Various fragments of HPK may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

Therapeutic and Diagnostic Uses of HPK Protein

The rationale for the use of nucleotide and polypeptide sequences disclosed herein is

based in part on the chemical and structural homology among the novel HPK and known protein kinases from C. elegans (GI 1082115), rat (GI 294637) and man (GI 1117791) (Wilson et al, supra; Webster et al. supra; Creasy et al. supra). Because of the widespread roles for protein kinases in cell signalling processes in various cells and tissues, altered HPK expression may be implicated in a variety of disorders and diseases.

HPK-1, by virtue of its occurrence in hippocampus, may be involved in memory and learning, and associated with disorders such as Alzheimers disease. Therefore, increasing HPK-1 activity through gene therapy using sequences encoding HPK-1 or by administering agonists of HPK-1 may be useful to reverse memory loss due to Alzheimers.

HPK-2 was identified in lymphocytes and associated with a variety of tumor tissues as well as with rheumatoid arthritis. HPK-2 may function in tumor promotion and may therefore provide a target for suppression by antisense molecules of sequences encoding HPK-2 or antagonists of HPK-2 activity as a cancer treatment strategy. Likewise, HPK-2 activity may promote the inflammatory response in arthritis conditions and again provide a target for suppression by antisense molecules of sequences encoding HPK-2 or antagonists of HPK-2 activity.

HPK-3 is derived from macrophages which suggests possible involvement in immune response or inflamation. The significant homology between HPK-3 and a glucocorticoid-regulated rat protein kinase, GI 294637, suggests that HPK-3 may be similarly regulated. HPK-3 expression may therefore be involved in the anti-inflammatory and immunosuppressive effects of glucocorticoid treatment for such conditions as asthma, multiple sclerosis, rheumatoid arthritis, as well as for certain cancers such as lymphocytic leukemias and lymphomas. Thus, increasing HPK-3 expression through gene therapy or through administration of agonists of HPK-3 may augument or provide an alternative to glucocorticoid treatment for these conditions.

HPK and/or a cell line that expresses HPK may be used to evaluate, screen and identify compounds, synthetic drugs, antibodies, peptides or other molecules that modulate the activity of HPK and may therefore be useful in the treatment of disease conditions associated with expression of HPK.

HPK Antibodies

HPK-specific antibodies may be useful for the diagnosis of conditions and diseases associated with expression of HPK. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by a Fab

expression library. Neutralizing antibodies.such as. those which inhibit dimer formation. are especially preferred for diagnostics and therapeutics.

For the production of antibodies. various hosts including goats. rabbits. rats. mice. etc may be immunized by injection with HPK or any portion. fragment or oligopeptide which retains immunogenic properties. It is not necessary that the protein fragment or oligopeptide used for antibody induction have a functional biological activity. however.it must be antigenic. Peptides used to induce specific antibodies may have an amino acid sequence consisting of at least five amino acids. preferably at least 10 amino acids. Preferably they should mimic a portion of the amino acid sequence of the natural protein and may contain the entire amino acid sequence of a small. naturally occurring molecule. Short stretches of HPK amino acids may be fused with those of another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule. Procedures well known in the art can be used for the production of antibodies to HPK.

Depending on the host species. various adjuvants may be used to increase immunological response. Such adjuvants include but are not limited to Freund's, mineral gels such as aluminum hydroxide. and surface active substances such as lysolecithin. pluronic polyols. polyanions. peptides. oil emulsions. keyhole limpet hemocyanin. and dinitrophenol. BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are potentially useful human adjuvants.

Monoclonal antibodies to HPK may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kochler and Milstein (1975 Nature 256:495-497). the human B-cell hybridoma technique (Kosbor et al (1983) Immunol Today 4:72; Cote et al (1983) Proc Natl Acad Sci 80:2026-2030) and the EBV-hybridoma technique (Cole et al (1985) Monoclonal Antibodies and Cancer Therapy, Alan R Liss Inc. New York NY. pp 77-96).

In addition. techniques developed for the production of "chimeric antibodies". the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison et al (1984) Proc Natl Acad Sci 81:6851-6855; Neuberger et al (1984) Nature 312:604-608; Takeda et al (1985) Nature 314:452-454). Alternatively, techniques described for the production of single chain antibodies (US Patent No. 4.946.778) can be adapted to produce HPK-specific single chain antibodies

Antibodies may also be produced by inducing in vivo production in the lymphocyte

population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi et al (1989, Proc Natl Acad Sci 86:3833-3837), and Winter G and Milstein C (1991: Nature 349:293-299).

Antibody fragments which contain specific binding sites for HPK may also be generated. For example, such fragments include, but are not limited to, the F(ab')2 fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse WD et al.(1989) Science 256:1275-1281).

A variety of protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the formation of complexes between HPK and its specific antibody and the measurement of complex formation. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on a specific HPK protein is preferred, but a competitive binding assay may also be employed. These assays are described in Maddox DE et al (1983, J Exp Med 158:1211).

Diagnostic Assays Using HPK Specific Antibodies

Particular HPK antibodies may be used for the diagnosis of conditions or diseases characterized by expression of HPK or in assays to monitor patients being treated with HPK agonists or antagonists. Diagnostic assays for HPK include methods utilizing the antibody and a label to detect HPK in human body fluids or extracts of cells or tissues. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, the polypeptides and antibodies will be labeled by joining them, either covalently or noncovalently, with a reporter molecule. A wide variety of reporter molecules are known, several of which are described above.

A variety of protocols for measuring HPK, using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HPK is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox, DE et al (1983, J Exp Med 158:1211).

In order to provide a basis for diagnosis, normal or standard values for HPK expression must be established. This is accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with antibody to HPK under conditions suitable for complex formation which are well known in the art. The amount of standard complex formation may be quantified by comparing various artificial membranes containing known quantities of HPK with both control and disease samples from biopsied tissues. Then, standard values obtained from normal samples may be compared with values obtained from samples from subjects symptomatic of the disease. Deviation between standard and subject values establishes the presence of a disease state.

Drug Screening

HPK, its catalytic or immunogenic fragments or oligopeptides thereof, can be used for screening therapeutic compounds in any of a variety of drug screening techniques. The fragment employed in such a test may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes, between HPK and the agent being tested, may be measured.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to HPK (WO Application 84/03564, incorporated herein by reference). In summary, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with fragments of HPK and washed. Bound HPK is then detected by methods well known in the art. Substantially purified HPK can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding HPK specifically compete with a test compound for binding HPK. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with HPK.

Diagnostic and Therapeutic Uses of the Polynucleotide Encoding HPK

A polynucleotide designated herein as an HPK encoding sequence, or any part thereof, may be used for diagnostic and/or therapeutic purposes. For diagnostic purposes, the HPK encoding sequences of this invention may be used to detect and quantitate gene expression in

biopsied tissues in which expression of HPK encoding sequences may be implicated. The diagnostic assay is useful to distinguish between absence, presence, and excess expression of HPK encoding sequences and to monitor regulation of HPK encoding sequences levels during therapeutic intervention. The association of HPK with disorders and disease conditions in specific tissues would greatly facilitate studies aimed at determining HPK function in these conditions and the development of therapeutic strategies to treat them. Included in the scope of the invention are oligonucleotide sequences, antisense RNA and DNA molecules, and PNAs.

In another embodimdent of the subject invention hybridization or PCR probesare provided which are capable of detecting polynucleotide sequences, including genomic sequences, encoding HPK or closely related molecules. The specificity of the probe, whether it is made from a highly specific region, eg, 10 unique nucleotides in the 5' regulatory region, or a less specific region, eg, especially in the 3' region, and the stringency of the hybridization or amplification (maximal, high, intermediate or low) will determine whether the probe identifies only naturally occurring HPK encoding sequences, alleles or related sequences.

Probes may also be used for the detection of related sequences and should preferably contain at least 50% of the nucleotides from any of these HPK encoding sequences. The hybridization probes of the subject invention may be derived from the nucleotide sequences of SEQ ID NOs:2, 4,and 6 or from genomic sequences including promoter, enhancer elements and introns of the naturally occurring HPK encoding sequences. Hybridization probes may be labeled by a variety of reporter groups, including radionuclides such as 32P or 35S, or enzymatic labels such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Other means for producing specific hybridization probes for HPK encoding sequences DNAs include the cloning of nucleic acid sequences encoding HPK or HPK derivatives into vectors for the production of mRNA probes. Such vectors are known in the art and are commercially available and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerase as T7 or SP6 RNA polymerase and the appropriate radioactively labeled nucleotides.

Polynucleotide sequences encoding HPK may be used for the diagnosis of conditions or diseases with which the expression of HPK is associated. For example, polynucleotide sequences encoding HPK may be used in hybridization or PCR assays of fluids or tissues from biopsies to detect HPK encoding sequences expression. The form of such qualitative or quantitative

methods may include southern or northern analysis, dot blot or other membrane-based technologies; PCR technologies; dip stick, pin, chip and ELISA technologies. All of these techniques are well known in the art and are the basis of many commercially available diagnostic kits.

The HPK encoding nucleotide sequences disclosed herein provide the basis for assays that detect activation or induction of HPK encoding sequences associated with specific diseases. The HPK encoding nucleotide sequence may be labeled by methods known in the art and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After an incubation period, the sample is washed with a compatible fluid which optionally contains a dye (or other label requiring a developer) if the nucleotide has been labeled with an enzyme. After the compatible fluid is rinsed off, the dye is quantitated and compared with a standard. If the amount of dye in the biopsied or extracted sample is significantly elevated over that of a comparable control sample, the nucleotide sequence has hybridized with nucleotide sequences in the sample, and the presence of elevated levels of HPK encoding nucleotide sequence in the sample indicates the presence of the associated disease.

Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regime in animal studies, in clinical trials, or in monitoring the treatment of an individual patient. In order to provide a basis for the diagnosis of disease, a normal or standard profile for HPK encoding sequence expression must be established. This is accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with an HPK encoding sequence, or a portion thereof, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained for normal subjects with a dilution series of an HPK encoding sequence run in the same experiment where a known amount of substantially purified HPK encoding sequence is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients afflicted with HPK-associated diseases. Deviation between standard and subject values is used to establish the presence of disease.

Once disease is established, a therapeutic agent is administered and a treatment profile is generated. Such assays may be repeated on a regular basis to evaluate whether the values in the profile progress toward or return to the normal or standard pattern. Successive treatment profiles may be used to show the efficacy of treatment over a period of several days or several months.

PCR may be used to provide additional uses for oligonucleotides based upon the HPK

sequence. Such oligomers are generally chemically synthesized, but they may be generated enzymatically or produced from a recombinant source. Oligomers generally comprise two nucleotide sequences, one with sense orientation (5'->3') and one with antisense (3'<-5'), employed under optimized conditions for identification of a specific gene or condition. The same two oligomers, nested sets of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for detection and/or quantitation of closely related DNA or RNA sequences.

Additionally, methods which may be used to quantitate the expression of a particular molecule include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated (Melby PC et al (1993) J Immunol Methods 159:235-44; Duplaa C et al (1993) Anal Biochem 229-36). Quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation. A definitive diagnosis of this type may allow health professionals to begin aggressive treatment and prevent further degeneration of the condition. Similarly, further assays can be used to monitor the progress of a patient during treatment. Furthermore, the nucleotide sequences disclosed herein may be used in molecular biology techniques that have not yet been developed, provided the new techniques rely on properties of nucleotide sequences that are currently known such as the triplet genetic code, specific base pair interactions, and the like.

For therapeutic purposes, an antisense molecule of an HPK encoding sequence may provide a basis for treatment where down-regulation of the gene and consequent inhibition of its activity is desirable. Alternatively, sequences encoding HPK may provide the basis for gene therapy in conditions where it may be desirable to increase expression of HPK and hence increase its activity.

Expression vectors derived from retroviruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue or cell population. Methods which are well known to those skilled in the art can be used to construct recombinant vectors which will express antisense HPK. See, for example, the techniques described in Sambrook et al (supra) and Ausubel et al (supra).

The polynucleotides comprising full length cDNA sequences encoding HPK and/or its regulatory elements may be used in research as an investigative tool in sense or antisense

regulation of gene function (Youssoufian H and HF Lodish 1993 Mol Cell Biol 13:98-104; Eguchi et al (1991) Annu Rev Biochem 60:631-652). Such technology is now well known in the art, and sense or antisense oligomers, or larger fragments, can be designed from various locations along the coding or control regions.

Genes encoding HPK can be turned off by transfecting a cell or tissue with expression vectors which express high levels of a desired HPK encoding sequence fragment. Such constructs can flood cells with untranslatable sense or antisense sequences. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until all copies are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector and even longer if appropriate replication elements are part of the vector system (Mettler I, personal communication).

As mentioned above, modifications of gene expression can be obtained by designing antisense molecules, DNA, RNA or PNA, to the control regions of HPK encoding sequences, ie, the promoters, enhancers, and introns. Oligonucleotides derived from the transcription initiation site, eg, between -10 and +10 regions of the leader sequence, are preferred. The antisense molecules may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA were reviewed by Gee JE et al (In: Huber BE and BI Carr (1994) Molecular and Immunologic Approaches, Futura Publishing Co, Mt Kisco NY).

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Another embodiment involves engineering hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of HPK encoding sequences. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization

with complementary oligonucleotides using ribonuclease protection assays.

Antisense molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding HPK. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly can be introduced into cell lines, cells or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine and wybutosine as well as acetyl-, methyl-, thio- and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Methods for introducing vectors into cells or tissues include those methods discussed infra and which are equally suitable for in vivo, in vitro and ex vivo therapy. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection and by liposome are quite well known in the art.

Furthermore, the nucleotide sequences for HPK encoding sequences disclosed herein may be used in molecular biology techniques that have not yet been developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including but not limited to such properties as the triplet genetic code and specific base pair interactions.

Detection and Mapping of Related Polynucleotide Sequences

The nucleic acid sequence for HPK can also be used to generate hybridization probes for mapping the naturally occurring genomic sequence. The sequence may be mapped to a particular chromosome or to a specific region of the chromosome using well known techniques. These include in situ hybridization to chromosomal spreads, flow-sorted chromosomal preparations, or artificial chromosome constructions such as yeast artificial chromosomes, bacterial artificial

chromosomes, bacterial P1 constructions or single chromosome cDNA libraries as reviewed in Price CM (1993; Blood Rev 7:127-34) and Trask BJ (1991; Trends Genet 7:149-54).

The technique of fluorescent in situ hybridization of chromosome spreads has been described, among other places, in Verma et al (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York NY. Fluorescent in situ hybridization of chromosomal preparations and other physical chromosome mapping techniques may be correlated with additional genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of an HPK encoding sequence on a physical chromosomal map and a specific disease (or predisposition to a specific disease) may help delimit the region of DNA associated with that genetic disease. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier or affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques such as linkage analysis using established chromosomal markers may be used for extending genetic maps. For example, a sequence tagged site based map of the human genome was recently published by the Whitehead-MIT Center for Genomic Research (Hudson TJ et al (1995) Science 270:1945-1954). Often the placement of a gene on the chromosome of another mammalian species such as mouse (Whitehead Institute/MIT Center for Genome Research, Genetic Map of the Mouse, Database Release 10, April 28, 1995) may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once a disease or syndrome, such as ataxia telangiectasia (AT), has been crudely localized by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti et al (1988) Nature 336:577-580), any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. among normal, carrier or affected individuals

Pharmaceutical Compositions

The present invention relates to pharmaceutical compositions which may comprise nucleotides, proteins, antibodies, agonists, antagonists, or inhibitors, alone or in combination with at least one other agent, such as stabilizing compound, which may be administered in any

sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. Any of these molecules can be administered to a patient alone, or in combination with other agents, drugs or hormones, in pharmaceutical compositions where it is mixed with excipient(s) or pharmaceutically acceptable carriers. In one embodiment of the present invention, the pharmaceutically acceptable carrier is pharmaceutically inert.

Administration of Pharmaceutical Compositions

Pharmaceutical compositions may be administerred to any subject in need of treatment including, but not limited to, humans and domestic animals. Administration of pharmaceutical compositions is accomplished orally or parenterally. Methods of parenteral delivery include topical, intra-arterial (directly to the tumor), intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal administration. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of "Remington's Pharmaceutical Sciences" (Maack Publishing Co, Easton PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; and gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores are provided with suitable coatings such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene

glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, ie, dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders such as lactose or starches, lubricants such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations for parenteral administration include aqueous solutions of active compounds. For injection, the pharmaceutical compositions of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Manufacture and Storage

The pharmaceutical compositions of the present invention may be manufactured in a manner that known in the art, eg, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder in 1mM-50 mM histidine, 0.1%-2% sucrose, 2%-7% mannitol at a pH range of 4.5 to 5.5 that is combined with buffer prior to use.

After pharmaceutical compositions comprising a compound of the invention formulated in a acceptable carrier have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of HPK, such labeling would include amount, frequency and method of administration.

Therapeutically Effective Dose

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, eg, of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of protein or its antibodies, antagonists, or inhibitors which ameliorate the symptoms or condition. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, eg, ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage is chosen by the individual physician in view of the patient to be treated. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Additional factors which may be taken into account include the severity of the disease state, eg, tumor size and location; age, weight and gender of the patient; diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long acting pharmaceutical compositions might be administered

every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery generally available in the scientific literature. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

It is contemplated, for example, that molecules or compounds that modulate HPK activity, such as antibodies of HPK, or an HPK derivative can be delivered in a suitable formulation as a therapeutic agent. Similarly, administration of agonists should also improve the activity or lifespan of this protein and lessen the onset and progression of senescence.

The examples below are provided to illustrate the subject invention and are not included for the purpose of limiting the invention.

INDUSTRIAL APPLICABILITY

I HPK-1 HIPONOTO1 cDNA Library Construction

The hippocampus used for this library was obtained from the Keystone Skin Bank, International Institute for the Advancement of Medicine (Exton, PA). Hippocampus tissue from 72 year old Caucasian female (RF94-09083) was flash frozen, ground in a mortar and pestle and lyzed immediately in buffer containing guanidinium isothiocyanate. Lysis was followed by several phenol chloroform extractions and ethanol precipitation. Poly A+ RNA was isolated using biotinylated oligo d(T) primer and streptavidin coupled to paramagnetic particles (Promega Corp, Madison WI) and sent to Stratagene. Stratagene prepared the cDNA library using oligo d(T) priming. Synthetic adapter oligonucleotides were ligated onto the cDNA molecules enabling them to be inserted into the Uni-ZAP™ vector system (Stratagene). The quality of the cDNA library was screened using DNA probes, and then the pBluescript phagemid (Stratagene) was excised. Subsequently, the custom-constructed library phage particles were infected into E. coli host strain XL1 Blue (Stratagene). Alternative unidirectional vectors might include, but are not limited to, pcDNAI (Invitrogen) and pSHlox-1 (Novagen).

The phagemid forms of individual cDNA clones were obtained by the in vivo excision process, in which the host bacterial strain was co-infected with both the library phage and an f1 helper phage. Polypeptides or enzymes derived from both the library-containing phage and the

helper phage nicked the DNA, initiated new DNA synthesis from defined sequences on the target DNA, and created a smaller, single stranded circular phagemid DNA molecule that included all DNA sequences of the pBluescript phagemid and the cDNA insert. The phagemid DNA was released from the cells and purified, and used to reinfect fresh host cells (SOLR, Stratagene) where double-stranded phagemid DNA was produced. Because the phagemid carries the gene for b-lactamase, the newly transformed bacteria were selected on medium containing ampicillin.

Phagemid DNA was purified using the QIAWELL-8 Plasmid Purification System from the QIAGEN DNA Purification System (QIAGEN Inc,Chatsworth,CA). The DNA was eluted from the purification resin and prepared for DNA sequencing and other analytical manipulations.

II HPK-2 TMLR30T01 cDNA Library Construction

The normal peripheral blood T-lymphocytes used for this library were obtained from two 24 year old, Caucasian males. This library represents a mixture of allogeneically stimulated human T cell populations obtained from Ficoll/Hypaque purified buffy coats. The cells from the two different donors (not typed for HLA alleles) were incubated at a density of 1 x 106/ml, cultured for 96 hours in DME containing 10% human serum, washed in PBS, scraped and lyzed immediately in buffer containing guanidinium isothiocyanate. The lysate was extracted twice with a mixture of phenol and chloroform, pH 8.0 and centrifuged over a CsCl cushion using an Beckman SW28 rotor in a L8-70M Ultracentrifuge (Beckman Instruments). The RNA was precipitated using 0.3 M sodium acetate and 2.5 volumes of ethanol, resuspended in water and DNase treated for 15 min at 37C. The total RNA was isolated using the Qiagen Oligotex kit (QIAGEN Inc, Chatsworth CA). It must be noted that B lymphocytes were not removed, and some contaminating macrophages may also have been present. Stratagene (La Jolla CA) used the total RNA to construct a custom cDNA library essentially as descibed above. The cDNAs were inserted into the LambdaZap™ vector system (Stratagene); and the vector was transformed into cells of E. coli, strain XL1-BlueMRF (Stratagene). The phagemid forms of individual cDNA clones were obtained by the in vivo excision process previously described.

Plasmid DNA was released from the cells and purified using the Miniprep Kit (Catalogue # 77468; Advanced Genetic Technologies Corporation, Gaithersburg MD), as previously described (Section V). Alternative methods of purifying plasmid DNA include the use of MAGIC MINIPREPS- DNA Purification System (Catalogue #A7100, Promega, Madison WI)or QIAwell–8 Plasmid, QIAwell PLUS DNA and QIAwell ULTRA DNA Purification Systems (QIAGEN Chatsworth CA).

III HPK-3 MPHGNOTO3 cDNA Library Construction

Peripheral blood was obtained from a 24 year old, Caucasian male. Mononuclear cells were separated from heparinized venous blood after centrifugation through Ficoll/Hypaque using HISTOPAQUE®-1119 and HISTOPAQUE®-1077, available from Sigma Diagnostics (St Louis MO). The Ficoll/Hypaque buffy coat which contains peripheral blood mononuclear cells was put into sterile Petri dishes and cultured for between 3 to 5 days in Dulbecco's minimum essential medium (DME) supplemented with 10% human serum. After incubation, macrophages mostly adhered to the plastic surface, whereas most other cell types, B and T lymphocytes, remained in solution. The DME was decanted from the wells and washed with phosphate buffered saline (PBS). Macrophages were released from the plastic surface by gently scraping the Petri dishes in PBS/1 mM EDTA. Macrophages were lysed immediately in buffer containing guanidinium isothiocyanate.

The lysate was extracted twice with a mixture of phenol and chloroform, pH 8.0 and centrifuged over a CsCl cushion using an Beckman SW28 rotor in a L8-70M Ultracentrifuge (Beckman Instruments). The RNA was precipitated using 0.3 M sodium acetate and 2.5 volumes of ethanol, resuspended in water and DNase treated for 15 min at 37%C. The total RNA was isolated using the Qiagen Oligotex kit (QIAGEN Inc, Chatsworth CA). It must be noted that some contaminating T and B lymphocytes may also have been present.

The poly A+ RNA was used to construct the MPHGNOTO3 cDNA library, phagemid forms of individual cDNA clones were obtained by the in vivo excision process, and plasmid DNA was released and recovered from the cells using the Miniprep Kit (Catalogue # 77468, Advanced Genetic Technologies Corporation, Gaithersburg MD), as described above.

IV Sequencing of cDNA Clones

The cDNAs were sequenced by the method of Sanger F and AR Coulson (1975; J Mol Biol 94:441f), using a Catalyst 800 Hamilton Micro Lab 2200 (Hamilton, Reno NV) in combination with four Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown MA) and Applied Biosystems 377 or 373 DNA Sequencing Systems (Perkin Elmer) and the reading frame was determined.

V Homology Searching of cDNA Clones and Their Deduced Proteins

Each cDNA was compared to sequences in GenBank using a search algorithm developed by Applied Biosystems and incorporated into the INHERIT™ 670 Sequence Analysis System. In this algorithm, Pattern Specification Language (TRW Inc. Los Angeles CA) was used to

determine regions of homology. The three parameters that determine how the sequence comparisons run were window size, window offset, and error tolerance. Using a combination of these three parameters, the DNA database was searched for sequences containing regions of homology to the query sequence, and the appropriate sequences were scored with an initial value. Subsequently, these homologous regions were examined using dot matrix homology plots to distinguish regions of homology from chance matches. Smith-Waterman alignments were used to display the results of the homology search.

Peptide and protein sequence homologies were ascertained using the INHERIT™ 670 Sequence Analysis System in a way similar to that used in DNA sequence homologies. Pattern Specification Language and parameter windows were used to search protein databases for sequences containing regions of homology which were scored with an initial value. Dot-matrix homology plots were examined to distinguish regions of significant homology from chance matches.

BLAST, which stands for Basic Local Alignment Search Tool (Altschul SF (1993) J Mol Evol 36:290-300; Altschul, SF et al (1990) J Mol Biol 215:403-10), was used to search for local sequence alignments. BLAST produces alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologs. BLAST is useful for matches which do not contain gaps. The fundamental unit of BLAST algorithm output is the High-scoring Segment Pair (HSP).

An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user. The BLAST approach is to look for HSPs between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The parameter E establishes the statistically significant threshold for reporting database sequence matches. E is interpreted as the upper bound of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search. Any database sequence whose match satisfies E is reported in the program output.

VI Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labelled nucleotide sequence to a membrane on which

RNAs from a particular cell type or tissue have been bound (Sambrook et al supra).

Analogous computer techniques using BLAST (Altschul SF 1993 and 1990, supra) are used to search for identical or related molecules in nucleotide databases such as GenBank or the LIFESEQ™ database (Incyte, Palo Alto CA). This analysis is much faster than multiple, membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or homologous.

The basis of the search is the product score which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

and it takes into acccount both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1-2% error; and at 70, the match will be exact. Homologous molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

VII Extension of HPK to Full Length or to Recover Regulatory Elements

The nucleic acid sequence of full length HPK encoding sequences (SEQ ID Nos:2, 4, or 6) is used to design oligonucleotide primers for extending a partial nucleotide sequence to full length or for obtaining 5' sequences from genomic libraries. One primer is synthesized to initiate extension in the antisense direction (XLR) and the other is synthesized to extend sequence in the sense direction (XLF).

Primers allow the extension of the known HPK encoding sequences "outward" generating amplicons containing new, unknown nucleotide sequences for the region of interest (US Patent Application 08/487,112). The initial primers are designed from the cDNA using OLIGO® 4.06 Primer Analysis Software (National Biosciences), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68%-72% C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations is avoided.

The original, selected cDNA libraries, or a human genomic library are used to extend the sequence; the latter is most useful to obtain 5' upstream regions. If more extension is necessary or desired, additional sets of primers are designed to further extend the known region.

By following the instructions for the XL-PCR kit (Perkin Elmer) and thoroughly mixing the enzyme and reaction mix, high fidelity amplification is obtained. Beginning with 40 pmol of

each primer and the recommended concentrations of all other components of the kit. PCR is performed using the Peltier Thermal Cycler (PTC200; MJ Research. Watertown MA) and the following parameters:

Step 1	94% C for 1 min (initial denaturation)
Step 2	65% C for 1 min
Step 3	68% C for 6 min
Step 4	94% C for 15 sec
Step 5	65% C for 1 min
Step 6	68% C for 7 min
Step 7	Repeat step 4-6 for 15 additional cycles
Step 8	94% C for 15 sec
Step 9	65% C for 1 min
Step 10	68% C for 7:15 min
Step 11	Repeat step 8-10 for 12 cycles
Step 12	72% C for 8 min
Step 13	4% C (and holding)

A 5-10 microliterl aliquot of the reaction mixture is analyzed by electrophoresis on a low concentration (about 0.6-0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. Bands thought to contain the largest products were selected and cut out of the gel. Further purification involves using a commercial gel extraction method such as QIAQuick™ (QIAGEN Inc). After recovery of the DNA, Klenow enzyme was used to trim single-stranded, nucleotide overhangs creating blunt ends which facilitate religation and cloning.

After ethanol precipitation. the products are redissolved in 13 microliter of ligation buffer. 1 microliter T4-DNA ligase (15 units) and 1 microliter T4 polynucleotide kinase are added. and the mixture is incubated at room temperature for 2-3 hours or overnight at 16% C. Competent E. coli cells (in 40 &l of appropriate media) are transformed with 3 microliter of ligation mixture and cultured in 80 &l of SOC medium (Sambrook J et al, supra). After incubation for one hour at 37% C, the whole transformation mixture is plated on Luria Bertani (LB)-agar (Sambrook J et al, supra) containing 2xCarb. The following day, several colonies are randomly picked from each plate and cultured in 150 microliter of liquid LB/2xCarb medium placed in an individual well of an appropriate, commercially-availablc, sterile 96-well microtiter plate. The following day, 5 microliter of each overnight culture is transferred into a non- sterile 96-well plate and after dilution 1:10 with water, 5 microliter of each sample is transferred into a PCR array.

For PCR amplification. 18 microliter of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer and one or both of the gene specific primers used for the extension reaction are added to each well. Amplification is performed using the

following conditions:

Step 1	94% C for 60 sec
Step 2	94% C for 20 sec
Step 3	55% C for 30 sec
Step 4	72% C for 90 sec
Step 5	Repeat steps 2-4 for an additional 29 cycles
Step 6	72% C for 180 sec
Step 7	4% C (and holding)

Aliquots of the PCR reactions are run on agarose gels together with molecular weight markers. The sizes of the PCR products are compared to the original partial cDNAs, and appropriate clones are selected, ligated into plasmid and sequenced.

VIII Labeling and Use of Hybridization Probes

Hybridization probes derived from SEQ ID NO:2 are employed to screen cDNAs, genomic DNAs or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base-pairs, is specifically described, essentially the same procedure is used with larger cDNA fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 (National Biosciences), labeled by combining 50 pmol of each oligomer and 250 mCi of [-^{32}P] adenosine triphosphate (Amersham, Chicago IL) and T4 polynucleotide kinase (DuPont NEN®, Boston MA). The labeled oligonucleotides are substantially purified with Sephadex G-25 super fine resin column (Pharmacia). A portion containing 10^7 counts per minute of each of the sense and antisense oligonucleotides is used in a typical membrane based hybridization analysis of human genomic DNA digested with one of the following endonucleases (Ase I, Bgl II, Eco RI, Pst I, Xba 1, or Pvu II; DuPont NEN®).

The DNA from each digest is fractionated on a 0.7 percent agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40%C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR™ film (Kodak, Rochester NY) is exposed to the blots in a Phosphoimager cassette (Molecular Dynamics, Sunnyvale CA) for several hours, hybridization patterns are compared visually.

IX Antisense Molecules

The HPK encoding sequence, or any part thereof, is used to inhibit in vivo or in vitro expression of naturally occurring HPK encoding sequences. Although use of antisense oligonucleotides, comprising about 20 base-pairs, is specifically described, essentially the same

procedure is used with larger cDNA fragments. For example, an oligonucleotide based on the coding sequence of HPK-1 as shown in Figures 1A, 1B, 1C and 1D is used to inhibit expression of naturally occurring HPK. The complementary oligonucleotide is designed from the most unique 5' sequence as shown in Figures 1A, 1B, 1C and 1D and used to inhibit translation of an HPK encoding sequences transcript by preventing the ribosome from binding. Using an appropriate portion of the leader and 5' sequence of SEQ ID NO:2, an effective antisense oligonucleotide includes any 15-20 nucleotides spanning the region which translates into the signal or early coding sequence of the polypeptide as shown in Figures 1A, 1B, 1C and 1D.

X Expression of HPK

Expression of the HPK is accomplished by subcloning the cDNAs into appropriate vectors and transfecting the vectors into host cells. In this case, the cloning vector, pSport, previously used for the generation of the cDNA library is used to express HPK in E. coli. Upstream of the cloning site, this vector contains a promoter for ß-galactosidase, followed by sequence containing the amino-terminal Met and the subsequent 7 residues of ß-galactosidase. Immediately following these eight residues is a bacteriophage promoter useful for transcription and a linker containing a number of unique restriction sites.

Induction of an isolated, transfected bacterial strain with IPTG using standard methods produces a fusion protein which consists of the first seven residues of ß-galactosidase, about 5 to 15 residues of linker, and the full length HPK. The signal sequence directs the secretion of HPK into the bacterial growth media which can be used directly in the following assay for activity.

XI HPK Activity

HPK activity may be measured by phosphorylation of a protein substrate using gamma-labeled ^{32}P-ATP and quantitation of the incorporated radioactivity using a gamma radioisotope counter. HPK is incubated with the protein substrate, ^{32}P-ATP, and a kinase buffer. The ^{32}P incorporated into the substrate is then separated from free ^{32}P-ATP by electrophoresis and the incorporated ^{32}P is counted. A determination of the specific amino acid residues phosphorylated is made by phosphoamino acid analysis of the hydrolyzed protein as described by Boyle WJ et al (1991) Methods in Enzymol 201: 110-148.

XII Production of HPK Specific Antibodies

HPK substantially purified using PAGE electrophoresis (Sambrook, supra) is used to immunize rabbits and to produce antibodies using standard protocols. The amino acid sequence translated from HPK is analyzed using DNAStar software (DNAStar Inc) to determine regions of

high immunogenicity and a corresponding oligopolypeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Analysis to select appropriate epitopes, such as those near the C-terminus or in hydrophilic regions (shown in Figures 4A, 4B, 4C and 4D) is described by Ausubel FM et al (supra).

Typically, the oligopeptides are 15 residues in length, synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry, and coupled to keyhole limpet hemocyanin (KLH, Sigma) by reaction with M-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS; Ausubel FM et al, supra). Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity, for example, by binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radioiodinated, goat anti-rabbit IgG.

XIII Purification of Naturally Occurring HPK Using Specific Antibodies

Naturally occurring or recombinant HPK is substantially purified by immunoaffinity chromatography using antibodies specific for HPK. An immunoaffinity column is constructed by covalently coupling HPK antibody to an activated chromatographic resin such as CnBr-activated Sepharose (Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing HPK is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of HPK (eg, high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/HPK binding (eg, a buffer of pH 2-3 or a high concentration of a chaotrope such as urea or thiocyanate ion), and HPK is collected.

XIV Identification of Molecules Which Interact with HPK

HPK, or biologically active fragments thereof, are labelled with ^{125}I Bolton-Hunter reagent (Bolton AE and Hunter WM (1973) Biochem J 133:529). Candidate molecules previously arrayed in the wells of a 96 well plate are incubated with the labelled HPK, washed and any wells with labelled HPK complex are assayed. Data obtained using different concentrations of HPK are used to calculate values for the number, affinity, and association of HPK with the candidate molecules.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit

of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: INCYTE PHARMACEUTICALS, INC.

(ii) TITLE OF THE INVENTION: NOVEL HUMAN PROTEIN KINASES

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Incyte Pharmaceuticals, Inc.
(B) STREET: 3174 Porter Drive
(C) CITY: Palo Alto
(D) STATE: CA
(E) COUNTRY: U.S.
(F) ZIP: 94304

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 1.5

(vi) CURRENT APPLICATION DATA:
(A) PCT APPLICATION NUMBER: To Be Assigned
(B) FILING DATE: Filed Herewith

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/712,709
(B) FILING DATE: Filed 12-SEP-1996

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Billings, Lucy J.
(B) REGISTRATION NUMBER: 36,749
(C) REFERENCE/DOCKET NUMBER: PF-0118 PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 650-855-0555
(B) TELEFAX: 650-845-4166

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 233 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE: Consensus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Met Met Asp Ala Lys Ala Lys Gln Asp Cys Val Lys Glu Ile Gly Leu
 1               5                  10                  15
Leu Lys Gln Leu Asn His Pro Asn Ile Ile Lys Tyr Leu Asp Ser Phe
            20                  25                  30
```

```
Ile Glu Asp Asn Glu Leu Asn Ile Val Leu Glu Leu Ala Asp Ala Gly
        35                  40                  45
Asp Leu Pro Gln Met Ile Lys Tyr Phe Lys Lys Gln Lys Arg Leu Ile
    50                  55                  60
Pro Glu Arg Thr Val Trp Lys Tyr Phe Val Gln Leu Cys Ser Ala Val
65                  70                  75                  80
Glu His Met His Ser Arg Arg Val Met His Arg Asp Ile Lys Pro Ala
                85                  90                  95
Asn Val Phe Ile Thr Ala Thr Gly Val Val Lys Leu Gly Asp Leu Gly
            100                 105                 110
Leu Gly Arg Phe Phe Ser Ser Glu Thr Thr Ala Ala His Ser Leu Val
        115                 120                 125
Gly Thr Pro Tyr Tyr Met Ser Pro Glu Arg Ile His Glu Asn Gly Tyr
    130                 135                 140
Asn Phe Lys Ser Asp Ile Trp Ser Leu Gly Cys Leu Leu Tyr Glu Met
145                 150                 155                 160
Ala Ala Leu Gln Ser Pro Phe Tyr Gly Asp Lys Met Asn Leu Phe Ser
                165                 170                 175
Leu Cys Gln Lys Ile Glu Gln Cys Asp Tyr Pro Pro Leu Pro Gly Glu
            180                 185                 190
His Tyr Ser Glu Lys Leu Arg Glu Leu Val Ser Met Cys Ile Cys Pro
        195                 200                 205
Asp Pro His Gln Arg Pro Asp Ile Gly Xaa Val His Gln Val Ala Lys
    210                 215                 220
Gln Met His Ile Trp Met Ser Ser Xaa
225                 230
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1347 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE: Consensus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
CATTCTGGGA CCTGTTCGCA GGACCGTCCG GTGTTCTGGC CCCCTGATGT CACCTTCACG     60
GGCCTGACTC ACAGTCCTAA ATATCTGACA GCGAAGATCG CTTGTAGTTC GTGCCCTCGT    120
GAGGCTGGCA TGCAGGATGG CAGGACAGCC CGGCCACATG CCCCATGGAG GGAGTTCCAA    180
CAACCTCTGC CACACCCTGG GGCCTGTGCA TCCTCCTGAC CCACAGAGGC ATCCCAACAC    240
GCTGTCTTTT CGCTGCTCGC TGGCGGACTT CCAGATCGAA AAGAAGATAG GCCGAGGACA    300
GTTCAGCGAG GTGTACAAGG CCACCTGCCT GCTGGACAGG AAGACAGTGG CTCTGRAGAA    360
GGTGCAGATC TTTGAGATGA TGGACGCCAA GGCGAAGCAG GACTGTGTCA AGGAGATCGG    420
CCTCTTGAAG CAACTGAACC ACCCAAATAT CATCAAGTAT TTGGACTCCT TTATCGAAGA    480
CAACGAACTG AACATTGTGC TGGAATTGGC TGACGCAGGG GACCTCCCGC AGATGATCAA    540
GTACTTTAAG AAGCAGAAGC GGCTCATCCC GGAGAGGACA GTATGGAAGT ACTTTGTGCA    600
GCTGTGCAGC GCCGTGGAGC ACATGCATTC ACGCCGGGTG ATGCACCGAG ACATCAAGCC    660
TGCCAACGTG TTCATCACAG CCACGGGCGT CGTGAAGCTC GGTGACCTTG GTCTGGGCCG    720
CTTCTTCAGC TCTGAAACCA CCGCAGCCCA CTCCCTAGTG GGGACGCCCT ACTACATGTC    780
ACCGGAGAGG ATCCATGAGA ACGGCTACAA CTTCAAGTCC GACATCTGGT CCTTGGGCTG    840
TCTGCTGTAC GAGATGGCAG CCCTCCAGAG CCCCTTCTAT GGAGATAAGA TGAATCTCTT    900
CTCCCTGTGC CAGAAGATCG AGCAGTGTGA CTACCCCCCA CTCCCCGGGG AGCACTACTC    960
CGAGAAGTTA CGAGAACTGG TCAGCATGTG CATCTGCCCT GACCCCCACC AGAGACCTGA   1020
CATCGGATAM GTGCACCAGG TGGCCAAGCA GATGCACATC TGGATGTCCA GCAMCTGAGC   1080
```

```
GTGGATGCAC CGTGCCTTAT CAAAGCCAGC ACCACTTTGC CTTACTTGAG TCGTCTTCTC   1140
TTCGAGTGGC CACCTGGTAG CCTAGAACAG CTAAGACCAC ANGNTTCAGC AGGTTCCCCA   1200
AAAGACTGCC CAGCCTTACA GCAGATGCTA AAGGNAGAGC AGCTGAGNGA GGGGCNCTNN   1260
CCACATNTCA CTGATGGTCA GATTCCAAAN TCCTTTCTTT ATACTGTTGT GGACAATCTC   1320
AGCTGGGTCA ATAAGGGCAG TTGGTTC                                       1347
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 403 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE: Consensus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Ala His Leu Arg Gly Phe Ala Asn Gln His Ser Arg Val Asp Pro
 1               5                  10                  15
Glu Glu Leu Phe Thr Lys Leu Asp Arg Ile Gly Lys Gly Ser Phe Gly
            20                  25                  30
Glu Val Tyr Lys Gly Ile Asp Asn His Thr Lys Glu Val Val Ala Ile
        35                  40                  45
Lys Ile Ile Asp Leu Glu Glu Ala Glu Asp Glu Ile Glu Asp Ile Gln
    50                  55                  60
Gln Glu Ile Thr Val Leu Ser Gln Cys Asp Ser Pro Tyr Ile Thr Arg
65                  70                  75                  80
Tyr Phe Gly Ser Tyr Leu Lys Ser Thr Lys Leu Trp Ile Ile Met Glu
                85                  90                  95
Tyr Leu Gly Gly Gly Ser Ala Leu Asp Leu Leu Lys Pro Gly Pro Leu
            100                 105                 110
Glu Glu Thr Tyr Ile Ala Thr Ile Leu Arg Glu Ile Leu Lys Gly Leu
        115                 120                 125
Asp Tyr Leu His Ser Glu Arg Lys Ile His Arg Asp Ile Lys Ala Ala
    130                 135                 140
Asn Val Leu Leu Ser Glu Gln Gly Asp Val Leu Ala Gly Gly Leu Trp
145                 150                 155                 160
Gly Ser Arg Gln Leu Thr Asp Thr Gln Ile Lys Arg Asn Thr Phe Val
                165                 170                 175
Gly Thr Pro Phe Trp Met Ala Pro Glu Val Ile Lys Gln Ser Ala Tyr
            180                 185                 190
Asp Phe Lys Ala Asp Ile Trp Ser Leu Gly Ile Thr Ala Ile Glu Leu
        195                 200                 205
Ala Lys Gly Glu Pro Pro Asn Ser Asp Leu His Pro Met Arg Val Leu
    210                 215                 220
Phe Leu Ile Pro Lys Asn Ser Pro Pro Thr Leu Glu Gly Gln His Ser
225                 230                 235                 240
Lys Pro Phe Lys Glu Phe Val Glu Ala Cys Leu Asn Lys Asp Pro Arg
                245                 250                 255
Phe Arg Pro Thr Ala Lys Glu Leu Leu Lys His Lys Phe Ile Thr Arg
            260                 265                 270
Tyr Thr Lys Lys Thr Ser Phe Leu Thr Glu Leu Ile Asp Arg Tyr Lys
        275                 280                 285
Arg Trp Lys Ser Glu Gly His Gly Glu Glu Ser Ser Ser Glu Asp Ser
    290                 295                 300
```

```
Asp Ile Asp Gly Glu Ala Glu Asp Gly Glu Gln Gly Pro Ile Trp Thr
305                 310                 315                 320
Phe Pro Pro Thr Ile Arg Pro Ser Pro His Ser Lys Leu His Lys Gly
                325                 330                 335
Thr Ala Leu His Ser Ser Gln Lys Pro Ala Glu Pro Val Lys Arg Gln
            340                 345                 350
Pro Arg Ser Gln Cys Leu Ser Thr Leu Val Arg Pro Val Phe Gly Glu
        355                 360                 365
Leu Lys Arg Ser Thr Ser Arg Ala Ala Gly Ala Trp Val Arg Trp Arg
    370                 375                 380
Ser Trp Arg Thr Pro Ser Ala Trp Pro Arg Ser Pro Ala Pro Ala Ser
385                 390                 395                 400
Gln Thr Ser
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2161 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE: Consensus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
CGTTAGGCCC GGGCGTGGCG GGGCCCCGGC GGCCTGGGGG GTCTCCTGGG CCCCCCCCCA     60
CCCATGGAGC CCGCCGCCCC GGAGGTCGGT CTCAGATGAC TGAACTGGGC ACCGAGCGCC    120
CCTGGTGTCC CTCGCAGTGG ACTGACGCCG CAGGGGCGAG CTAGCCGGCT CCGCGCCTCT    180
CCGCGGGATC CAGACGNCTC CTGGGGCTGC TGGCGGAGGG TCTGACGCGG CGCGGCCATG    240
GCTCACCTCC GGGGATTTGC CAACCAGCAC TCTCGAGTGG ACCCTGAGGA GCTCTTCACC    300
AAGCTCGACC GCATTGGCAA GGGCTCGTTT GGGGAGGTCT ACAAGGGCAT CGATAACCAC    360
ACAAAGGAGG TGGTGGCCAT CAAGATCATC GACCTGGAGG AGGCCGAGGA TGAGATCGAG    420
GACATCCAGC AGGAGATCAC TGTCCTCAGT CAGTGCGACA GCCCCTACAT CACCCGCTAC    480
TTTGGCTCCT ACCTAAAGAG CACCAAGCTA TGGATCATCA TGGAGTACCT GGGCGGCGGC    540
TCAGCACTGG ACTTGCTTAA ACCAGGTCCC CTGGAGGAGA CATACATTGC CACGATCCTG    600
CGGGAGATTC TGAAGGGCCT GGATTATCTG CACTCCGAAC GCAAGATCCA CCGAGACATC    660
AAAGCTGCCA ACGTGCTACT CTCGGAGCAG GGTGACGTGT TAGCTGGCGG ACTTTGGGGT    720
AGCAGGCAGC TCACAGACAC GCAGATTAAG AGGAACACAT TCGTGGGCAC CCCCTTCTGG    780
ATGGCACCTG AGGTCATCAA GCAGTCGGCC TACGACTTCA AGGCTGACAT CTGGTCCCTG    840
GGGATCACAG CCATCGAGCT CGCCAAGGGG GAGCCTCCAA ACTCTGACCT CCACCCCATG    900
CGCGTCCTGT TCCTGATTCC CAAGAACAGC CCACCCACAC TGGAGGGCCA GCACAGCAAG    960
CCCTTCAAGG AGTTCGTGGA GGCCTGCCTC AACAAAGACC CCCGATTCCG GCCCACGGCC   1020
AAGGAGCTCC TGAAGCACAA GTTCATCACA CGCTACACCA AGAAGACCTC CTTCCTCACG   1080
GAGCTCATCG ACCGCTATAA GCGCTGGAAG TCAGAGGGGC ATGGCGAGGA GTCCAGCTCT   1140
GAGGACTCTG ACATTGATGG CGAGGCGGAG GACGGGGAGC AGGGCCCCAT CTGGACGTTC   1200
CCCCCTACCA TCCGGCCGAG TCCACACAGC AAGCTTCACA AGGGGACGGC CCTGCACAGT   1260
TCACAGAAGC CTGCGGAGCC CGTCAAGAGG CAGCCGAGGT CCCAGTGCCT GTCCACGCTG   1320
GTCCGGCCCG TTTTCGGAGA GCTCAAGAGA AGCACAAGCA GAGCGGCGGG AGCGTGGGTG   1380
CGCTGGAGGA GCTGGAGAAC GCCTTCAGCC TGGCCGAGGA GTCCTGCCCC GGCATCTCAG   1440
ACAAGCTGAT GGTGCACCTG GTGGAGCGAG TGCAGAGGTT TTCACACAAC AGAAACCACC   1500
TGACATCCAC CCGCTGAAGC GCACTGCTGT TCAGATAGGG GACGGAAGGT CGTTTGTTTT   1560
TGTTCTGAGC TCCATAAGAA CTGTGCTGAC TTGGAAGGTG CCCTGTGCTA TGTCGTGCCT   1620
GCAGGGACAC GTCGGATCCC GTGGGCCTCA CATGCCAGGT CACCAGGTCA CCGTCTCCTT   1680
CCACCCCTGC AGTGTGCTGT TGTGCACGTC AGGGACGCTG TTCTCTATGC CCACTGCCCT   1740
CCTCCCTCTC CTGGCCCAGC AGTATTGCTC ACGGGGGCTC CAGCCGCCGG CGTGGCCCTC   1800
```

```
ATGAGCTACG CCTGGGTCTT CTGCAGACTC ATGCAGCCCT ATGGCCGCTC AGACCAAGGC   1860
GCAGAGCAAC TATCAGGGCA GCTCTGCCTC CTCCTCCCAT GAGGTGGGGA GAGGCAACAG   1920
GGCAGCCCCC AGAGGAGTGT CCTGGCCGCT GTCCTCCCGG GGCCCATGAT GGCCATAGAT   1980
TTGCCTTGTG GTGTTGGATC AGGTACTGTG TCTGCTCATA AGTACTTGTG TCATCCAGAA   2040
TGTTTTGTTT TTTAAGAAAA TTGAATTACT TGTTTCCTGA AATATTCTGA GGTTAATATG   2100
TTAGTTTTCA TAGAACATTG AGAGGCCCCT GCCACTTTCA ATAAAGACCT GACTTGGAGN   2160
C                                                                   2161
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 431 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE: Consensus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5.

```
Met Ala Val Lys Thr Glu Ala Ala Lys Gly Thr Leu Thr Tyr Ser Arg
 1               5                  10                  15
Met Arg Gly Met Val Ala Ile Leu Ile Ala Phe Met Lys Gln Arg Arg
            20                  25                  30
Met Gly Leu Asn Asp Phe Ile Gln Lys Ile Ala Asn Asn Ser Tyr Ala
        35                  40                  45
Cys Lys His Pro Glu Val Gln Ser Ile Leu Lys Ile Ser Gln Pro Gln
    50                  55                  60
Glu Pro Glu Leu Met Asn Ala Asn Pro Ser Pro Pro Pro Ser Pro Ser
65                  70                  75                  80
Gln Gln Ile Asn Leu Gly Pro Ser Ser Asn Pro His Ala Lys Pro Ser
                85                  90                  95
Asp Phe His Phe Leu Lys Val Ile Gly Lys Gly Ser Phe Gly Lys Val
            100                 105                 110
Leu Leu Ala Arg His Lys Ala Glu Glu Val Phe Tyr Ala Val Lys Val
        115                 120                 125
Leu Gln Lys Lys Ala Ile Leu Lys Lys Lys Glu Glu Lys His Ile Met
    130                 135                 140
Ser Glu Arg Asn Val Leu Leu Lys Asn Val Lys His Pro Phe Leu Val
145                 150                 155                 160
Gly Leu His Phe Ser Phe Gln Thr Ala Asp Lys Leu Tyr Phe Val Leu
                165                 170                 175
Asp Tyr Ile Asn Gly Gly Glu Leu Phe Tyr His Leu Gln Arg Glu Arg
            180                 185                 190
Cys Phe Leu Glu Pro Arg Ala Arg Ser Tyr Ala Ala Glu Ile Ala Ser
        195                 200                 205
Ala Leu Gly Tyr Leu His Ser Leu Asn Ile Val Tyr Arg Asp Leu Lys
    210                 215                 220
Pro Glu Asn Ile Leu Leu Asp Ser Gln Gly His Ile Val Leu Thr Asp
225                 230                 235                 240
Phe Gly Leu Cys Lys Glu Asn Ile Glu His Asn Ser Thr Thr Ser Thr
                245                 250                 255
Phe Cys Gly Thr Pro Glu Tyr Leu Ala Pro Glu Val Leu His Lys Gln
            260                 265                 270
Pro Tyr Asp Arg Thr Val Asp Trp Trp Cys Leu Gly Ala Val Leu Tyr
        275                 280                 285
```

```
Glu Met Leu Tyr Gly Leu Pro Pro Phe Tyr Ser Arg Asn Thr Ala Glu
    290                 295                 300
Met Tyr Asp Asn Ile Leu Asn Lys Pro Leu Gln Leu Lys Pro Asn Ile
305                 310                 315                 320
Thr Asn Ser Ala Arg His Leu Leu Glu Gly Leu Leu Gln Lys Asp Arg
                325                 330                 335
Thr Lys Arg Leu Gly Ala Lys Asp Asp Phe Met Glu Ile Lys Ser His
            340                 345                 350
Val Phe Phe Ser Leu Ile Asn Trp Asp Asp Leu Ile Asn Lys Lys Ile
        355                 360                 365
Thr Pro Pro Phe Asn Pro Asn Val Ser Gly Pro Asn Asp Leu Arg His
    370                 375                 380
Phe Asp Pro Glu Phe Thr Glu Glu Pro Val Pro Asn Ser Ile Gly Lys
385                 390                 395                 400
Ser Pro Asp Ser Val Leu Val Thr Ala Ser Val Lys Glu Ala Ala Glu
                405                 410                 415
Ala Phe Leu Gly Phe Ser Tyr Ala Pro Pro Thr Asp Ser Phe Leu
            420                 425                 430
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2311 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(A) LIBRARY:
(B) CLONE: Consensus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
GCGGTGGTGA TGGCGGTGAA AACTGAGGCT GCTAAGGGCA CCCTCACTTA CTCCAGGATG      60
AGGGGCATGG TGGCAATTCT CATCGCTTTC ATGAAGCAGA GGAGGATGGG TCTGAACGAC     120
TTTATTCAGA AGATTGCCAA TAACTCCTAT GCATGCAAAC ACCCTGAAGT TCAGTCCATC     180
TTGAAGATCT CCCAACCTCA GGAGCCTGAG CTTATGAATG CCAACCCTTC TCCTCCACCA     240
AGTCCTTCTC AGCAAATCAA CCTTGGCCCG TCGTCCAATC CTCATGCTAA ACCATCTGAC     300
TTTCACTTCT TGAAAGTGAT CGGAAAGGGC AGTTTTGGAA AGGTTCTTCT AGCAAGACAC     360
AAGGCAGAAG AAGTGTTCTA TGCAGTCAAA GTTTTACAGA AGAAAGCAAT CCTGAAAAAG     420
AAAGAGGAGA AGCATATTAT GTCGGAGCGG AATGTTCTGT TGAAGAATGT GAAGCACCCT     480
TTCCTGGTGG GCCTTCACTT CTCTTTCCAG ACTGCTGACA AATTGTACTT TGTCCTAGAC     540
TACATTAATG GTGGAGAGTT GTTCTACCAT CTCCAGAGGG AACGCTGCTT CCTGGAACCA     600
CGGGCTCGTT CCTATGCTGC TGAAATAGCC AGTGCCTTGG GCTACCTGCA TTCACTGAAC     660
ATCGTTTATA GAGACTTAAA ACCAGAGAAT ATTTTGCTAG ATTCACAGGG ACACATTGTC     720
CTTACTGACT TCGGACTCTG CAAGGAGAAC ATTGAACACA ACAGCACAAC ATCCACCTTC     780
TGTGGCACGC CGGAGTATCT CGCACCTGAG GTGCTTCATA AGCAGCCTTA TGACAGGACT     840
GTGGACTGGT GGTGCCTGGG AGCTGTCTTG TATGAGATGC TGTATGGCCT GCCGCCTTTT     900
TATAGCCGAA ACACAGCTGA AATGTACGAC AACATTCTGA ACAAGCCTCT CCAGCTGAAA     960
CCAAATATTA CAAATTCCGC AAGACACCTC CTGGAGGGCC TCCTGCAGAA GGACAGGACA    1020
AAGCGGCTCG GGGCCAAGGA TGACTTCATG GAGATTAAGA GTCATGTCTT CTTCTCCTTA    1080
ATTAACTGGG ATGATCTCAT TAATAAGAAG ATTACTCCCC CTTTTAACCC AAATGTGAGT    1140
GGGCCCAACG ACCTACGGCA CTTTGACCCC GAGTTTACCG AAGAGCCTGT CCCCAACTCC    1200
ATTGGCAAGT CCCCTGACAG CGTCCTCGTC ACAGCCAGCG TCAAGGAAGC TGCCGAGGCT    1260
TTCCTAGGCT TTTCCTATGC GCCTCCCACG GACTCTTTCC TCTGAACCCT GTTAGGGCTT    1320
GGTTTTAAAG GATTTTATGT GTGTTTCCGA ATGTTTTAGT TAGCCTTTTG GTGGAGCCGC    1380
CAGCTGACAG GACATCTTAC AAGAGAATTT GCACATCTCT GGAAGCTTAG CAATCTTATT    1440
GCACACTGTT CGCTGGAAGC TTTTTGAAGA GCACATTCTC CTCAGTGAGC TCATGAGGTT    1500
```

```
TTCATTTTTA TTCTTCCTTC CAACGTGGTG CTATCTCTGA AACGAGCGTT AGAGTGCCGC   1560
CTTAGACGGA GGCAGGAGTT TCGTTAGAAA GCGGACGCTG TTCTAAAAAA GGTCTCCTGC   1620
AGATCTGTCT GGGCTGTGAT GACGAATATT ATGAAATGTG CCTTTTCTGA AGAAAATTGT   1680
GTTAGCTCCA AAGCTTTTCC TATCGCAGTG TTTCAGTTCT TTATTTTCCC TTGTGGATAT   1740
GCTGTGTGAA CCGTCGTGTG AGTGTGGTAT GCCTGATCAC AGATGGATTT TGTTATAAGC   1800
ATCAATGTGA CACTTGCAGG ACACTACAAC GTGGGACATT GTTTGTTTCT TCCATATTTG   1860
GAAGATAAAT TTATGTGTAG ACTTTTTTGT AAGATACGGT TAATAACTAA AATTTATTGA   1920
AATGGTCTTG CAATGACTCG TATTCAGATG CTTAAAGAAA GCATTGCTGC TACAAATATT   1980
TCTATTTTTA GAAAGGGTTT TTATGGACCA ATGCCCCAGT TGTCAGTCAG AGCCGTTGGT   2040
GTTTTTCATT GTTTAAAATG TCACCTGTAA AATGGGCATT ATTTATGTTT TTTTTTTGC    2100
ATTCCTGATA ATTGTATGTA TTGTATAAAG AACGTCTGTA CATTGGGTTA TAACACTAGT   2160
ATATTTAAAC TTACAGGCTT ATTTGTAATG TAAACCACCA TTTTAATGTA CTGTAATTAA   2220
CATGGTTATA ATACGNACAA TCCTTCCCTC ATCCCATCAC ACAACTTTTT TTGTGTGTGA   2280
TAAACTGATT TTGGTTTGCA ATAAAACCTT G                                  2311
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 239 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: GenBank
(B) CLONE: 1082115

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Val Phe Glu Met Val Asp Gln Lys Ala Arg Gln Asp Cys Leu Lys Glu
 1               5                  10                  15
Ile Asp Leu Leu Lys Gln Leu Asn His Val Asn Val Ile Arg Tyr Tyr
            20                  25                  30
Ala Ser Phe Ile Asp Asn Asn Gln Leu Asn Ile Val Leu Glu Leu Ala
        35                  40                  45
Glu Ala Gly Asp Met Ser Arg Met Ile Lys His Phe Lys Lys Gly Gly
    50                  55                  60
Arg Leu Ile Pro Glu Lys Thr Ile Trp Lys Tyr Phe Val Gln Leu Ala
65                  70                  75                  80
Arg Ala Leu Ala His Met His Ser Lys Arg Ile Met His Arg Asp Ile
                85                  90                  95
Lys Pro Ala Asn Val Phe Ile Thr Gly Asn Gly Ile Val Lys Leu Gly
            100                 105                 110
Asp Leu Gly Leu Gly Arg Phe Phe Ser Ser Lys Thr Thr Ala Ala His
        115                 120                 125
Ser Leu Val Gly Thr Pro Tyr Tyr Met Ser Pro Glu Arg Ile Gln Glu
    130                 135                 140
Ser Gly Tyr Asn Phe Lys Ser Asp Leu Trp Ser Thr Gly Cys Leu Leu
145                 150                 155                 160
Tyr Glu Met Ala Ala Leu Gln Ser Pro Phe Tyr Gly Asp Lys Met Asn
                165                 170                 175
Leu Tyr Ser Leu Cys Lys Lys Ile Glu Asn Cys Glu Tyr Pro Pro Leu
            180                 185                 190
Pro Ala Asp Ile Tyr Ser Thr Gln Val Ser Ala Asn Leu Cys Phe Val
        195                 200                 205
Gln Leu Ser Ser Ala Thr Trp Tyr Pro Val Val Tyr Phe Gln Lys Leu
    210                 215                 220
Gln Asn Asp Gln Arg Pro Val Lys Phe Tyr Arg Phe Val Pro Arg
225                 230                 235
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 487 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: GenBank
(B) CLONE: 1117791

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Glu Thr Val Gln Leu Arg Asn Pro Pro Arg Arg Gln Leu Lys Lys
 1               5                  10                  15
Leu Asp Glu Asp Ser Leu Thr Lys Gln Pro Glu Glu Val Phe Asp Val
            20                  25                  30
Leu Glu Lys Leu Gly Glu Gly Ser Tyr Gly Ser Val Tyr Lys Ala Ile
        35                  40                  45
His Lys Glu Thr Gly Gln Ile Val Ala Ile Lys Gln Val Pro Val Glu
    50                  55                  60
Ser Asp Leu Gln Glu Ile Ile Lys Glu Ile Ser Ile Met Gln Gln Cys
65                  70                  75                  80
Asp Ser Pro His Val Val Lys Tyr Tyr Gly Ser Tyr Phe Lys Asn Thr
                85                  90                  95
Asp Leu Trp Ile Val Met Glu Tyr Cys Gly Ala Gly Ser Val Ser Asp
            100                 105                 110
Ile Ile Arg Leu Arg Asn Lys Thr Leu Thr Glu Asp Glu Ile Ala Thr
        115                 120                 125
Ile Leu Gln Ser Thr Leu Lys Gly Leu Glu Tyr Leu His Phe Met Arg
    130                 135                 140
Lys Ile His Arg Asp Ile Lys Ala Gly Asn Ile Leu Leu Asn Thr Glu
145                 150                 155                 160
Gly His Ala Lys Leu Ala Asp Phe Gly Val Ala Gly Gln Leu Thr Asp
                165                 170                 175
Thr Met Ala Lys Arg Asn Thr Val Ile Gly Thr Pro Phe Trp Met Ala
            180                 185                 190
Pro Glu Val Ile Gln Glu Ile Gly Tyr Asn Cys Val Ala Asp Ile Trp
        195                 200                 205
Ser Leu Gly Ile Thr Ala Ile Glu Met Ala Glu Gly Lys Arg Pro Tyr
    210                 215                 220
Ala Asp Ile His Pro Met Arg Ala Ile Phe Met Ile Pro Thr Asn Pro
225                 230                 235                 240
Pro Pro Thr Phe Arg Lys Pro Glu Leu Trp Ser Asp Asn Phe Thr Asp
                245                 250                 255
Phe Val Lys Gln Cys Leu Val Lys Ser Pro Glu Gln Arg Ala Thr Ala
            260                 265                 270
Thr Gln Leu Leu Gln His Pro Phe Val Arg Ser Ala Lys Gly Val Ser
        275                 280                 285
Ile Leu Arg Asp Leu Ile Asn Glu Ala Met Asp Val Lys Leu Lys Arg
    290                 295                 300
Gln Glu Ser Gln Gln Arg Glu Met Asp Gln Asp Asp Glu Glu Asn Ser
305                 310                 315                 320
Glu Glu Asp Glu Met Asp Ser Gly Thr Met Val Arg Ala Val Gly Asp
                325                 330                 335
Glu Met Gly Thr Val Arg Val Ala Ser Thr Met Thr Asp Gly Ala Asn
            340                 345                 350
```

```
Thr Met Ile Glu His Asp Asp Thr Leu Pro Ser Gln Leu Gly Thr Met
        355                 360                 365
Val Ile Asn Ala Glu Asp Glu Glu Glu Gly Thr Met Lys Arg Arg
    370                 375                 380
Asp Glu Thr Met Gln Pro Ala Lys Pro Ser Phe Leu Glu Tyr Phe Glu
385                 390                 395                 400
Gln Lys Glu Lys Glu Asn Gln Ile Asn Ser Phe Gly Lys Ser Val Pro
                405                 410                 415
Gly Pro Leu Lys Asn Ser Ser Asp Trp Lys Ile Pro Gln Asp Gly Asp
            420                 425                 430
Tyr Glu Phe Leu Lys Ser Trp Thr Val Glu Asp Leu Gln Lys Arg Leu
        435                 440                 445
Leu Ala Leu Asp [illegible] Met Met Glu Gln Glu Ile Glu Glu [illegible] Arg Gln
    450                 455                 460
Lys Tyr Gln Ser Lys Arg Gln Pro Ile Leu Asp Ala Ile Glu Ala Lys
465                 470                 475                 480
Lys Arg Arg Gln Gln Asn Phe
                485
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 430 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: GenBank
(B) CLONE: 294637

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Met Thr Val Lys Thr Glu Ala Ala Arg Ser Thr Leu Thr Tyr Ser Arg
 1               5                  10                  15
Met Arg Gly Met Val Ala Ile Leu Ile Ala Phe Met Lys Gln Arg Arg
            20                  25                  30
Met Gly Leu Asn Asp Phe Ile Gln Lys Leu Ala Asn Asn Ser Tyr Ala
        35                  40                  45
Cys Lys His Pro Glu Val Gln Ser Tyr Leu Lys Ile Ser Gln Pro Gln
    50                  55                  60
Glu Pro Glu Leu Met Asn Ala Asn Pro Ser Pro Pro Pro Ser Pro Ser
65                  70                  75                  80
Gln Gln Ile Asn Leu Gly Pro Ser Ser Asn Pro His Ala Lys Pro Ser
                85                  90                  95
Asp Phe His Phe Leu Lys Val Ile Gly Lys Gly Ser Phe Gly Lys Val
            100                 105                 110
Leu Leu Ala Arg His Lys Ala Glu Glu Ala Phe Tyr Ala Val Lys Val
        115                 120                 125
Leu Gln Lys Lys Ala Ile Leu Lys Lys Lys Glu Glu Lys His Ile Met
    130                 135                 140
Ser Glu Arg Asn Val Leu Leu Lys Asn Val Lys His Pro Phe Leu Val
145                 150                 155                 160
Gly Leu His Phe Ser Phe Gln Thr Ala Asp Lys Leu Tyr Phe Val Leu
                165                 170                 175
Asp Tyr Ile Asn Gly Gly Glu Leu Phe Tyr His Leu Gln Arg Glu Arg
            180                 185                 190
```

```
Cys Phe Leu Glu Pro Arg Ala Arg Phe Tyr Ala Ala Glu Ile Ala Ser
        195                 200                 205
Ala Leu Gly Tyr Leu His Ser Leu Asn Ile Val Tyr Arg Asp Leu Lys
    210                 215                 220
Pro Glu Asn Ile Leu Leu Asp Ser Gln Gly His Ile Val Leu Thr Asp
225                 230                 235                 240
Phe Gly Leu Cys Lys Glu Asn Ile Glu His Asn Gly Thr Thr Ser Thr
                245                 250                 255
Phe Cys Gly Thr Pro Glu Tyr Leu Ala Pro Glu Val Leu His Lys Gln
            260                 265                 270
Pro Tyr Asp Arg Thr Val Asp Trp Trp Cys Leu Gly Ala Val Leu Tyr
        275                 280                 285
Glu Met Leu Tyr Gly Leu Pro Pro Phe Tyr Ser Arg Asn Thr Ala Glu
    290                 295                 300
Met Tyr Asp Asn Ile Leu Asn Lys Pro Leu Gln Leu Lys Asn Ile Thr
305                 310                 315                 320
Asn Ser Ala Arg His Leu Leu Glu Gly Leu Leu Gln Lys Asp Arg Thr
                325                 330                 335
Lys Arg Leu Gly Ala Lys Asp Asp Phe Met Glu Ile Lys Ser His Ile
            340                 345                 350
Phe Phe Ser Leu Ile Asn Trp Asp Asp Leu Ile Asn Lys Lys Ile Thr
        355                 360                 365
Pro Pro Phe Asn Pro Asn Val Ser Gly Pro Ser Asp Leu Arg His Phe
    370                 375                 380
Asp Pro Glu Phe Thr Glu Glu Pro Val Pro Ser Ser Ile Gly Arg Ser
385                 390                 395                 400
Pro Asp Ser Ile Leu Val Thr Ala Ser Val Lys Glu Ala Ala Glu Ala
                405                 410                 415
Phe Leu Gly Phe Ser Tyr Ala Pro Pro Met Asp Ser Phe Leu
            420                 425                 430
```

What is claimed is:

1. A substantially purified human protein kinase polypeptide comprising the amino acid sequence of SEQ ID NO:1 or fragments thereof.

2. The isolated and purified polynucleotide sequence encoding the polypeptide of claim 1.

3. The isolated and purified polynucleotide sequence of claim 2 comprising the sequence of SEQ ID NO:2 or variants thereof.

4. A polynucleotide sequence which is complementary to the sequence of SEQ ID NO:2 or variants thereof.

5. A recombinant expression vector comprising the polynucleotide sequence of claim 2.

6. A recombinant host cell comprising the expression vector of claim 5.

7. A method for producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO:1. the method comprising the steps of:

a) culturing the host cell of claim 6 under conditions suitable for the expression of the polypeptide; and

b) recovering the polypeptide from the host cell culture.

8. A pharmaceutical composition comprising a substantially purified human protein kinase polypeptide having the amino acid sequence of SEQ ID NO:1 or fragments thereof in conjunction with a suitable pharmaceutical carrier.

9. A purified antibody which binds specifically to the polypeptide of claim 1.

10. A purified antagonist which specifically regulates or modulates the activity of the polypeptide of claim 1.

11. A pharmaceutical composition comprising a substantially purified antagonist of the polypeptide of claim 1 in conjunction with a suitable pharmaceutical carrier.

12. A substantially purified human protein kinase polypeptide comprising the amino acid sequence of SEQ ID NO:3 or fragments thereof.

13. The isolated and purified polynucleotide sequence encoding the polypeptide of claim 12.

14. The isolated and purified polynucleotide sequence of claim 13 comprising the sequence of SEQ ID NO:4 or variants thereof.

15. A polynucleotide sequence which is complementary to the sequence of SEQ ID NO:4 or variants thereof.

16. A recombinant expression vector comprising the polynucleotide sequence of claim 13.

17. A recombinant host cell comprising the expression vector of claim 16.

18. A method for producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO:3, the method comprising the steps of:

a) culturing the host cell of claim 17 under conditions suitable for the expression of the polypeptide; and

b) recovering the polypeptide from the host cell culture.

19. A pharmaceutical composition comprising a substantially purified human protein kinase polypeptide having the amino acid sequence of SEQ ID NO:3 or fragments thereof in conjunction with a suitable pharmaceutical carrier.

20. A purified antibody which binds specifically to the polypeptide of claim 12.

21. A purified antagonist which specifically regulates or modulates the activity of the polypeptide of claim 12.

22. A pharmaceutical composition comprising a substantially purified antagonist of the polypeptide of claim 12 in conjunction with a suitable pharmaceutical carrier.

23. A substantially purified human protein kinase polypeptide comprising the amino acid sequence of SEQ ID NO:5 or fragments thereof.

24. The isolated and purified polynucleotide sequence encoding the polypeptide of claim 23.

25. The isolated and purified polynucleotide sequence of claim 24 comprising the sequence of SEQ ID NO:6 or variants thereof.

26. A polynucleotide sequence which is complementary to the sequence of SEQ ID NO:6 or variants thereof.

27. A recombinant expression vector comprising the polynucleotide sequence of claim 24.

28. A recombinant host cell comprising the expression vector of claim 27.

29. A method for producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO:5, the method comprising the steps of:

a) culturing the host cell of claim 28 under conditions suitable for the expression of the polypeptide; and

b) recovering the polypeptide from the host cell culture.

30. A pharmaceutical composition comprising a substantially purified human protein kinase polypeptide having the amino acid sequence of SEQ ID NO:5 or fragments thereof in conjunction with a suitable pharmaceutical carrier.

31. A purified antibody which binds specifically to the polypeptide of claim 23.

32. A purified antagonist which specifically regulates or modulates the activity of the polypeptide of claim 23.

33. A pharmaceutical composition comprising a substantially purified antagonist of the polypeptide of claim 23 in conjunction with a suitable pharmaceutical carrier.

```
             9          18          27          36          45          54
5' NNC ATT CTG GGA CCT GTT CGC AGG ACC GTC CGG TGT TCT GGC CCC CTG ATG TCA

            63          72          81          90          99         108
CCT TCA CGG GCC TGA CTC ACA GTC CTA AAT ATC TGA CAG CGA AGA TCG CTT GTA

           117         126         135         144         153         162
GTT CGT GCC CTC GTG AGG CTG GCA TGC AGG ATG GCA GGA CAG CCC GGC CAC ATG

           171         180         189         198         207         216
CCC CAT GGA GGG AGT TCC AAC AAC CTC TGC CAC ACC CTG GGG CCT GTG CAT CCT

           225         234         243         252         261         270
CCT GAC CCA CAG AGG CAT CCC AAC ACG CTG TCT TTT CGC TGC TCG CTG GCG GAC

           279         288         297         306         315         324
TTC CAG ATC GAA AAG AAG ATA GGC CGA GGA CAG TTC AGC GAG TG TAC AAG GCC

           333         342         351         360         369         378
ACC TGC CTG CTG GAC AGG AAG ACA GTG GCT CTG RAG AAG GTG CAG ATC TTT GAG

           387         396         405         414         423         432
ATG ATG GAC GCC AAG GCG AAG CAG GAC TGT GTC AAG GAG ATC GGC CTC TTG AAG
M   M   D   A   K   A   K   Q   D   C   V   K   E   I   G   L   L   K
```

FIGURE 1A

```
         441        450        459        468        477        486
CAA CTG AAC CAC CCA AAT ATC ATC AAG TAT TTG GAC TCC TTT ATC GAA GAC AAC
Q   L   N   H   P   N   I   I   K   Y   L   D   S   F   I   E   D   N

         495        504        513        522        531        540
GAA CTG AAC ATT GTG CTG GAA TTG GCT GAC GCA GGG GAC CTC CCG CAG ATG ATC
E   L   N   I   V   L   E   L   A   D   A   G   D   L   P   Q   M   I

         549        558        567        576        585        594
AAG TAC TTT AAG AAG CAG AAG CGG CTC ATC CCG GAG AGG ACA GTA TGG AAG TAC
K   Y   F   K   K   Q   K   R   L   I   P   E   R   T   V   W   K   Y

         603        612        621        630        639        648
TTT GTG CAG CTG TGC AGC GCC GTG GAG CAC ATG CAT TCA CGC CGG GTG ATG CAC
F   V   Q   L   C   S   A   V   E   H   M   H   S   R   R   V   M   H

         657        666        675        684        693        702
CGA GAC ATC AAG CCT GCC AAC GTG TTC ATC ACA GCC ACG GGC GTC GTG AAG CTC
R   D   I   K   P   A   N   V   F   I   T   A   T   G   V   V   K   L

         711        720        729        738        747        756
GGT GAC CTT GGT CTG GGC CGC TTC TTC AGC TCT GAA ACC ACC GCA GCC CAC TCC
G   D   L   G   L   G   R   F   F   S   S   E   T   T   A   A   H   S

         765        774        783        792        801        810
CTA GTG GGG ACG CCC TAC TAC ATG TCA CCG GAG AGG ATC CAT GAG AAC GGC TAC
L   V   G   T   P   Y   Y   M   S   P   E   R   I   H   E   N   G   Y
```

FIGURE 1B

```
         819         828         837         846         855         864
AAC TTC AAG TCC GAC ATC TGG TCC TTG GGC TGT CTG CTG TAC GAG ATG GCA GCC
N   F   K   S   D   I   W   S   L   G   C   L   L   Y   E   M   A   A

         873         882         891         900         909         918
CTC CAG AGC CCC TTC TAT GGA GAT AAG ATG AAT CTC TTC TCC CTG TGC CAG AAG
L   Q   S   P   F   Y   G   D   K   M   N   L   F   S   L   C   Q   K

         927         936         945         954         963         972
ATC GAG CAG TGT GAC TAC CCC CCA CTC CCC GGG GAG CAC TAC TCC GAG AAG TTA
I   E   Q   C   D   Y   P   P   L   P   G   E   H   Y   S   E   K   L

         981         990         999        1008        1017        1026
CGA GAA CTG GTC AGC ATG TGC ATC TGC CCT GAC CCC CAC CAG AGA CCT GAC ATC
R   E   L   V   S   M   C   I   C   P   D   P   H   Q   R   P   D   I

        1035        1044        1053        1062        1071        1080
GGA TAM GTG CAC CAG GTG GCC AAG CAG ATG CAC ATC TGG ATG TC  AGC AMC TGA
G   X   V   H   Q   V   A   K   Q   M   H   I   W   M   S   S   X

        1089        1098        1107        1116        1125        1134
GCG TGG ATG CAC CGT GCC TTA TCA AAG CCA GCA CCA CTT TGC CTT ACT TGA GTC

        1143        1152        1161        1170        1179        1188
GTC TTC TCT TCG AGT GGC CAC CTG GTA GCC TAG AAC AGC TAA GAC CAC ANG NTT
```

FIGURE 1C

```
   1197        1206        1215        1224        1233        1242
CAG CAG GTT CCC CAA AAG ACT GCC CAG CCT TAC AGC AGA TGC TAA AGG NAG AGC

       1251        1260        1269        1278        1287        1296
AGC TGA GNG AGG GGC NCT NNC CAC ATN TCA CTG ATG GTC AGA TTC CAA ANT CCT

       1305        1314        1323        1332        1341
TTC TTT ATA CTG TTG TGG ACA ATC TCA GCT GGG TCA ATA AGG GCA GTT GGT TC 3'
```

FIGURE 1D

```
            9          18          27          36          45          54
5' CGT TAG GCC CGG GCG TGG CGG GGC CCC GGC GGC CTG GGG GGT CTC CTG GGC CCC

           63          72          81          90          99         108
   CCC CCA CCC ATG GAG CCC GCC GCC CCG GAG GTC GGT CTC AGA TGA CTG AAC TGG

          117         126         135         144         153         162
   GCA CCG AGC GCC CCT GGT GTC CCT CGC AGT GGA CTG ACG CCG CAG GGG CGA GCT

          171         180         189         198         207         216
   AGC CGG CTC CGC GCC TCT CCG CGG GAT CCA GAC GNC TCC TGG GGC TGC TGG CGG

          225         234         243         252         261         270
   AGG GTC TGA CGC GGC GCG GCC ATG GCT CAC CTC CGG GGA TTT GCC AAC CAG CAC
                                  M   A   H   L   R   G   F   A   N   Q   H

          279         288         297         306         315         324
   TCT CGA GTG GAC CCT GAG GAG CTC TTC ACC AAG CTC GAC CGC ATT GGC AAG GGC
   S   R   V   D   P   E   E   L   F   T   K   L   D   R   I   G   K   G

          333         342         351         360         369         378
   TCG TTT GGG GAG GTC TAC AAG GGC ATC GAT AAC CAC ACA AAG GAG GTG GTG GCC
   S   F   G   E   V   Y   K   G   I   D   N   H   T   K   E   V   V   A

          387         396         405         414         423         432
   ATC AAG ATC ATC GAC CTG GAG GAG GCC GAG GAT GAG ATC GAG GAC ATC CAG CAG
   I   K   I   I   D   L   E   E   A   E   D   E   I   E   D   I   Q   Q
```

FIGURE 2A

```
    441         450         459         468         477         486
GAG ATC ACT GTC CTC AGT CAG TGC GAC AGC CCC TAC ATC ACC CGC TAC TTT GGC
E   I   T   V   L   S   Q   C   D   S   P   Y   I   T   R   Y   F   G

        495         504         513         522         531         540
TCC TAC CTA AAG AGC ACC AAG CTA TGG ATC ATC ATG GAG TAC CTG GGC GGC GGC
S   Y   L   K   S   T   K   L   W   I   I   M   E   Y   L   G   G   G

        549         558         567         576         585         594
TCA GCA CTG GAC TTG CTT AAA CCA GGT CCC CTG GAG GAG ACA TAC ATT GCC ACG
S   A   L   D   L   L   K   P   G   P   L   E   E   T   Y   I   A   T

        603         612         621         630         639         648
ATC CTG CGG GAG ATT CTG AAG GGC CTG GAT TAT CTG CAC TCC GAA CGC AAG ATC
I   L   R   E   I   L   K   G   L   D   Y   L   H   S   E   R   K   I

        657         666         675         684         693         702
CAC CGA GAC ATC AAA GCT GCC AAC GTG CTA CTC TCG GAG CAG GGT GAC GTG TTA
H   R   D   I   K   A   A   N   V   L   L   S   E   Q   G   D   V   L

        711         720         729         738         747         756
GCT GGC GGA CTT TGG GGT AGC AGG CAG CTC ACA GAC ACG CAG ATT AAG AGG AAC
A   G   G   L   W   G   S   R   Q   L   T   D   T   Q   I   K   R   N

        765         774         783         792         801         810
ACA TTC GTG GGC ACC CCC TTC TGG ATG GCA CCT GAG GTC ATC AAG CAG TCG GCC
T   F   V   G   T   P   F   W   M   A   P   E   V   I   K   Q   S   A
```

FIGURE 2B

```
         819          828          837          846          855          864
TAC GAC TTC AAG GCT GAC ATC TGG TCC CTG GGG ATC ACA GCC ATC GAG CTC GCC
Y   D   F   K   A   D   I   W   S   L   G   I   T   A   I   E   L   A

         873          882          891          900          909          918
AAG GGG GAG CCT CCA AAC TCT GAC CTC CAC CCC ATG CGC GTC CTG TTC CTG ATT
K   G   E   P   P   N   S   D   L   H   P   M   R   V   L   F   L   I

         927          936          945          954          963          972
CCC AAG AAC AGC CCA CCC ACA CTG GAG GGC CAG CAC AGC AAG CCC TTC AAG GAG
P   K   N   S   P   P   T   L   E   G   Q   H   S   K   P   F   K   E

         981          990          999         1008         1017         1026
TTC GTG GAG GCC TGC CTC AAC AAA GAC CCC CGA TTC CGG CCC ACG GCC AAG GAG
F   V   E   A   C   L   N   K   D   P   R   F   R   P   T   A   K   E

        1035         1044         1053         1062         1071         1080
CTC CTG AAG CAC AAG TTC ATC ACA CGC TAC ACC AAG AAG ACC TCC TTC CTC ACG
L   L   K   H   K   F   I   T   R   Y   T   K   K   T   S   F   L   T

        1089         1098         1107         1116         1125         1134
GAG CTC ATC GAC CGC TAT AAG CGC TGG AAG TCA GAG GGG CAT GGC GAG GAG TCC
E   L   I   D   R   Y   K   R   W   K   S   E   G   H   G   E   E   S

        1143         1152         1161         1170         1179         1188
AGC TCT GAG GAC TCT GAC ATT GAT GGC GAG GCG GAG GAC GGG GAG CAG GGC CCC
S   S   E   D   S   D   I   D   G   E   A   E   D   G   E   Q   G   P
```

FIGURE 2C

```
      1197        1206        1215        1224        1233        1242
ATC TGG ACG TTC CCC CCT ACC ATC CGG CCG AGT CCA CAC AGC AAG CTT CAC AAG
I   W   T   F   P   P   T   I   R   P   S   P   H   S   K   L   H   K

      1251        1260        1269        1278        1287        1296
GGG ACG GCC CTG CAC AGT TCA CAG AAG CCT GCG GAG CCC GTC AAG AGG CAG CCG
G   T   A   L   H   S   S   Q   K   P   A   E   P   V   K   R   Q   P

      1305        1314        1323        1332        1341        1350
AGG TCC CAG TGC CTG TCC ACG CTG GTC CGG CCC GTT TTC GGA GAG CTC AAG AGA
R   S   Q   C   L   S   T   L   V   R   P   V   F   G   E   L   K   R

      1359        1368        1377        1386        1395        1404
AGC ACA AGC AGA GCG GCG GGA GCG TGG GTG CGC TGG AGG AGC TGG AGA ACG CCT
S   T   S   R   A   A   G   A   W   V   R   W   R   S   W   R   T   P

      1413        1422        1431        1440        1449        1458
TCA GCC TGG CCG AGG AGT CCT GCC CCG GCA TCT CAG ACA AGC TGA TGG TGC ACC
S   A   W   P   R   S   P   A   P   A   S   Q   T   S

      1467        1476        1485        1494        1503        1512
TGG TGG AGC GAG TGC AGA GGT TTT CAC ACA ACA GAA ACC ACC TGA CAT CCA CCC

      1521        1530        1539        1548        1557        1566
GCT GAA GCG CAC TGC TGT TCA GAT AGG GGA CGG AAG GTC GTT TGT TTT TGT TCT
```

FIGURE 2D

```
      1575        1584        1593        1602        1611        1620
GAG CTC CAT AAG AAC TGT GCT GAC TTG GAA GGT GCC CTG TGC TAT GTC GTG CCT

      1629        1638        1647        1656        1665        1674
GCA GGG ACA CGT CGG ATC CCG TGG GCC TCA CAT GCC AGG TCA CCA GGT CAC CGT

      1683        1692        1701        1710        1719        1728
CTC CTT CCA CCC CTG CAG TGT GCT GTT GTG CAC GTC AGG GAC GCT GTT CTC TAT

      1737        1746        1755        1764        1773        1782
GCC CAC TGC CCT CCT CCC TCT CCT GGC CCA GCA GTA TTG CTC ACG GGG GCT CCA

      1791        1800        1809        1818        1827        1836
GCC GCC GGC GTG GCC CTC ATG AGC TAC GCC TGG GTC TTC TGC AGA CTC ATG CAG

      1845        1854        1863        1872        1881        1890
CCC TAT GGC CGC TCA GAC CAA GGC GCA GAG CAA CTA TCA GGG CAG CTC TGC CTC

      1899        1908        1917        1926        1935        1944
CTC CTC CCA TGA GGT GGG GAG AGG CAA CAG GGC AGC CCC CAG AGG AGT GTC CTG

      1953        1962        1971        1980        1989        1998
GCC GCT GTC CTC CCG GGG CCC ATG ATG GCC ATA GAT TTG CCT TGT GGT GTT GGA

      2007        2016        2025        2034        2043        2052
TCA GGT ACT GTG TCT GCT CAT AAG TAC TTG TGT CAT CCA GAA TGT TTT GTT TTT
```

FIGURE 2E

```
  2061        2070        2079        2088        2097        2106
TAA GAA AAT TGA ATT ACT TGT TTC CTG AAA TAT TCT GAG GTT AAT ATG TTA GTT

       2115        2124        2133        2142        2151        2160
TTC ATA GAA CAT TGA GAG GCC CCT GCC ACT TTC AAT AAA GAC C G ACT TGG AGN

C 3'
```

FIGURE 2F

```
                9          18          27          36          45          54
5' GCG GTG GTG ATG GCG GTG AAA ACT GAG GCT GCT AAG GGC ACC CTC ACT TAC TCC
                M   A   V   K   T   E   A   A   K   G   T   L   T   Y   S

               63          72          81          90          99         108
   AGG ATG AGG GGC ATG GTG GCA ATT CTC ATC GCT TTC ATG AAG CAG AGG AGG ATG
   R   M   R   G   M   V   A   I   L   I   A   F   M   K   Q   R   R   M

              117         126         135         144         153         162
   GGT CTG AAC GAC TTT ATT CAG AAG ATT GCC AAT AAC TCC TAT GCA TGC AAA CAC
   G   L   N   D   F   I   Q   K   I   A   N   N   S   Y   A   C   K   H

              171         180         189         198         207         216
   CCT GAA GTT CAG TCC ATC TTG AAG ATC TCC CAA CCT CAG GAG CCT GAG CTT ATG
   P   E   V   Q   S   I   L   K   I   S   Q   P   Q   E   P   E   L   M

              225         234         243         252         261         270
   AAT GCC AAC CCT TCT CCT CCA CCA AGT CCT TCT CAG CAA ATC AA  CTT GGC CCG
   N   A   N   P   S   P   P   P   S   P   S   Q   Q   I   N   L   G   P

              279         288         297         306         315         324
   TCG TCC AAT CCT CAT GCT AAA CCA TCT GAC TTT CAC TTC TTG AAA GTG ATC GGA
   S   S   N   P   H   A   K   P   S   D   F   H   F   L   K   V   I   G

              333         342         351         360         369         378
   AAG GGC AGT TTT GGA AAG GTT CTT CTA GCA AGA CAC AAG GCA GAA GAA GTG TTC
   K   G   S   F   G   K   V   L   L   A   R   H   K   A   E   E   V   F
```

FIGURE 3A

```
    387         396         405         414         423         432
TAT GCA GTC AAA GTT TTA CAG AAG AAA GCA ATC CTG AAA AAG AAA GAG GAG AAG
Y   A   V   K   V   L   Q   K   K   A   I   L   K   K   K   E   E   K

        441         450         459         468         477         486
CAT ATT ATG TCG GAG CGG AAT GTT CTG TTG AAG AAT GTG AAG CAC CCT TTC CTG
H   I   M   S   E   R   N   V   L   L   K   N   V   K   H   P   F   L

        495         504         513         522         531         540
GTG GGC CTT CAC TTC TCT TTC CAG ACT GCT GAC AAA TTG TAC TTT GTC CTA GAC
V   G   L   H   F   S   F   Q   T   A   D   K   L   Y   F   V   L   D

        549         558         567         576         585         594
TAC ATT AAT GGT GGA GAG TTG TTC TAC CAT CTC CAG AGG GAA CGC TGC TTC CTG
Y   I   N   G   G   E   L   F   Y   H   L   Q   R   E   R   C   F   L

        603         612         621         630         639         648
GAA CCA CGG GCT CGT TCC TAT GCT GCT GAA ATA GCC AGT GCC TTG GGC TAC CTG
E   P   R   A   R   S   Y   A   A   E   I   A   S   A   L   G   Y   L

        657         666         675         684         693         702
CAT TCA CTG AAC ATC GTT TAT AGA GAC TTA AAA CCA GAG AAT ATT TTG CTA GAT
H   S   L   N   I   V   Y   R   D   L   K   P   E   N   I   L   L   D

        711         720         729         738         747         756
TCA CAG GGA CAC ATT GTC CTT ACT GAC TTC GGA CTC TGC AAG GAG AAC ATT GAA
S   Q   G   H   I   V   L   T   D   F   G   L   C   K   E   N   I   E
```

FIGURE 3B

```
         765          774          783          792          801          810
CAC AAC AGC ACA ACA TCC ACC TTC TGT GGC ACG CCG GAG TAT CTC GCA CCT GAG
H   N   S   T   T   S   T   F   C   G   T   P   E   Y   L   A   P   E

         819          828          837          846          855          864
GTG CTT CAT AAG CAG CCT TAT GAC AGG ACT GTG GAC TGG TGG TGC CTG GGA GCT
V   L   H   K   Q   P   Y   D   R   T   V   D   W   W   C   L   G   A

         873          882          891          900          909          918
GTC TTG TAT GAG ATG CTG TAT GGC CTG CCG CCT TTT TAT AGC CGA AAC ACA GCT
V   L   Y   E   M   L   Y   G   L   P   P   F   Y   S   R   N   T   A

         927          936          945          954          963          972
GAA ATG TAC GAC AAC ATT CTG AAC AAG CCT CTC CAG CTG AAA CCA AAT ATT ACA
E   M   Y   D   N   I   L   N   K   P   L   Q   L   K   P   N   I   T

         981          990          999         1008         1017         1026
AAT TCC GCA AGA CAC CTC CTG GAG GGC CTC CTG CAG AAG GAC AGG ACA AAG CGG
N   S   A   R   H   L   L   E   G   L   L   Q   K   D   R   T   K   R

        1035         1044         1053         1062         1071         1080
CTC GGG GCC AAG GAT GAC TTC ATG GAG ATT AAG AGT CAT GTC TTC TTC TCC TTA
L   G   A   K   D   D   F   M   E   I   K   S   H   V   F   F   S   L

        1089         1098         1107         1116         1125         1134
ATT AAC TGG GAT GAT CTC ATT AAT AAG AAG ATT ACT CCC CCT TTT AAC CCA AAT
I   N   W   D   D   L   I   N   K   K   I   T   P   P   F   N   P   N
```

FIGURE 3C

```
       1143        1152        1161        1170        1179        1188
GTG AGT GGG CCC AAC GAC CTA CGG CAC TTT GAC CCC GAG TTT ACC GAA GAG CCT
V   S   G   P   N   D   L   R   H   F   D   P   E   F   T   E   E   P

       1197        1206        1215        1224        1233        1242
GTC CCC AAC TCC ATT GGC AAG TCC CCT GAC AGC GTC CTC GTC ACA GCC AGC GTC
V   P   N   S   I   G   K   S   P   D   S   V   L   V   T   A   S   V

       1251        1260        1269        1278        1287        1296
AAG GAA GCT GCC GAG GCT TTC CTA GGC TTT TCC TAT GCG CCT CCC ACG GAC TCT
K   E   A   A   E   A   F   L   G   F   S   Y   A   P   P   T   D   S

       1305        1314        1323        1332        1341        1350
TTC CTC TGA ACC CTG TTA GGG CTT GGT TTT AAA GGA TTT TAT GTG TGT TTC CGA
F   L

       1359        1368        1377        1386        1395        1404
ATG TTT TAG TTA GCC TTT TGG TGG AGC CGC CAG CTG ACA GGA CAT CTT ACA AGA

       1413        1422        1431        1440        1449        1458
GAA TTT GCA CAT CTC TGG AAG CTT AGC AAT CTT ATT GCA CAC TGT TCG CTG GAA

       1467        1476        1485        1494        1503        1512
GCT TTT TGA AGA GCA CAT TCT CCT CAG TGA GCT CAT GAG GTT TTC ATT TTT ATT

       1521        1530        1539        1548        1557        1566
CTT CCT TCC AAC GTG GTG CTA TCT CTG AAA CGA GCG TTA GAG TGC CGC CTT AGA
```

FIGURE 3D

```
      1575        1584        1593        1602        1611        1620
CGG AGG CAG GAG TTT CGT TAG AAA GCG GAC GCT GTT CTA AAA AAG GTC TCC TGC

      1629        1638        1647        1656        1665        1674
AGA TCT GTC TGG GCT GTG ATG ACG AAT ATT ATG AAA TGT GCC TTT TCT GAA GAA

      1683        1692        1701        1710        1719        1728
AAT TGT GTT AGC TCC AAA GCT TTT CCT ATC GCA GTG TTT CAG TTC TTT ATT TTC

      1737        1746        1755        1764        1773        1782
CCT TGT GGA TAT GCT GTG TGA ACC GTC GTG TGA GTG TGG TAT GCC TGA TCA CAG

      1791        1800        1809        1818        1827        1836
ATG GAT TTT GTT ATA AGC ATC AAT GTG ACA CTT GCA GGA CAC TAC AAC GTG GGA

      1845        1854        1863        1872        1881        1890
CAT TGT TTG TTT CTT CCA TAT TTG GAA GAT AAA TTT ATG TGT AGA CTT TTT TGT

      1899        1908        1917        1926        1935        1944
AAG ATA CGG TTA ATA ACT AAA ATT TAT TGA AAT GGT CTT GCA ATG ACT CGT ATT

      1953        1962        1971        1980        1989        1998
CAG ATG CTT AAA GAA AGC ATT GCT GCT ACA AAT ATT TCT ATT TTT AGA AAG GGT

      2007        2016        2025        2034        2043        2052
TTT TAT GGA CCA ATG CCC CAG TTG TCA GTC AGA GCC GTT GGT GTT TTT CAT TGT
```

FIGURE 3E

```
       2061        2070        2079        2088        2097        2106
TTA AAA TGT CAC CTG TAA AAT GGG CAT TAT TTA TGT TTT TTT TTT TGC ATT CCT

       2115        2124        2133        2142        2151        2160
GAT AAT TGT ATG TAT TGT ATA AAG AAC GTC TGT ACA TTG GGT TAT AAC ACT AGT

       2169        2178        2187        2196        2205        2214
ATA TTT AAA CTT ACA GGC TTA TTT GTA ATG TAA ACC ACC ATT TTA ATG TAC TGT

       2223        2232        2241        2250        2259        2268
AAT TAA CAT GGT TAT AAT ACG NAC AAT CCT TCC CTC ATC CCA TCA CAC AAC TTT

       2277        2286        2295        2304
TTT TGT GTG TGA TAA ACT GAT TTT GGT TTG CAA TAA AAC CTT G 3'
```


FIGURE 3F

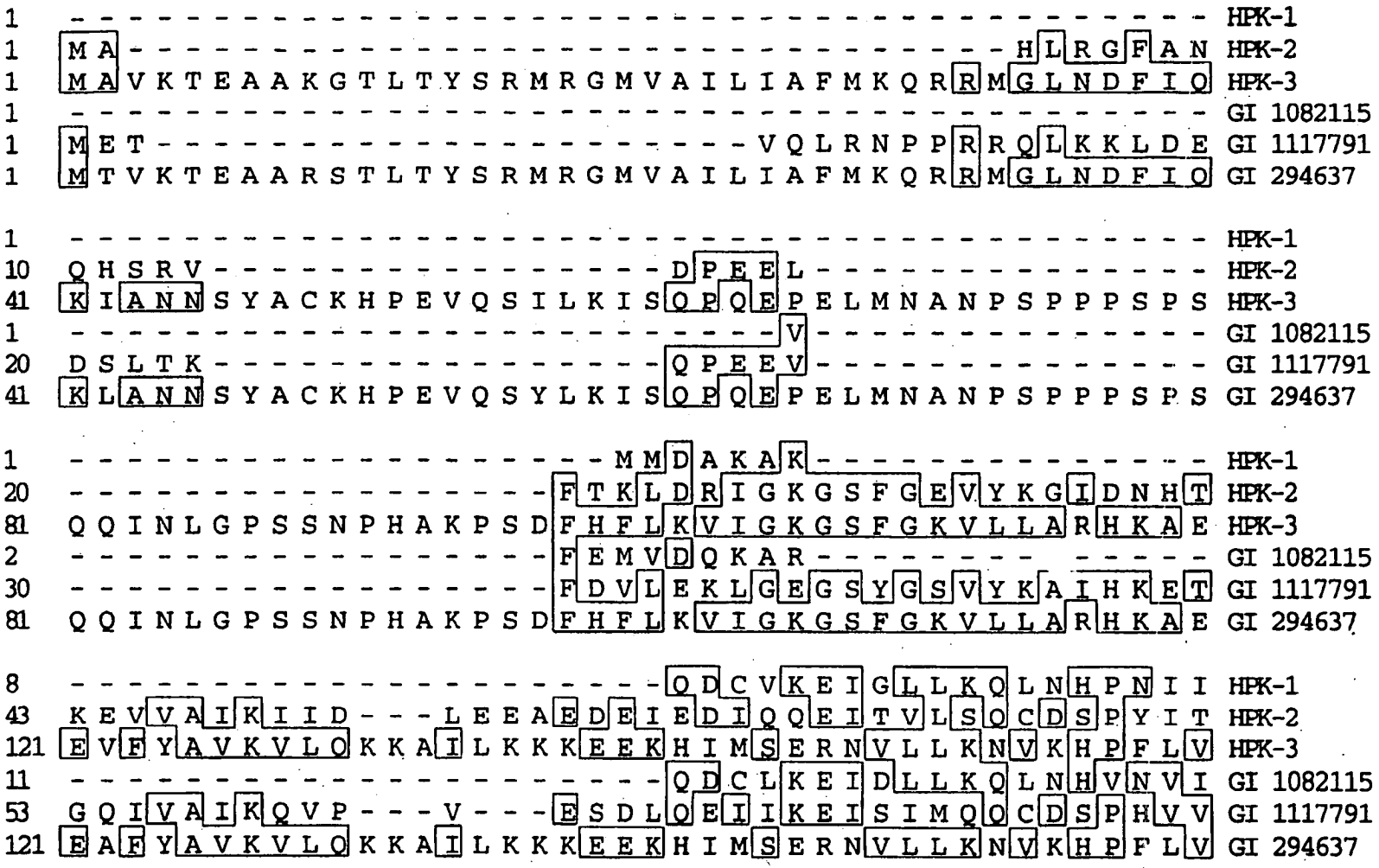
1 HPK-1
1 MA HLRGFAN HPK-2
1 MAVKTEAAKGTLTYSRMRGMVAILIAFMKQRRMGLNDFIQ HPK-3
1 GI 1082115
1 METVQLRNPPRRQLKKLDE GI 1117791
1 MTVKTEAARSTLTYSRMRGMVAILIAFMKQRRMGLNDFIQ GI 294637
1 HPK-1
10 QHSRVDPEEL HPK-2
41 KIANNSYACKHPEVQSILKISQPQEPELMNANPSPPPSPS HPK-3
1 V GI 1082115
20 DSLTKQPEEV GI 1117791
41 KLANNSYACKHPEVQSYLKISQPQEPELMNANPSPPPSPS GI 294637
1 MMDAKAK HPK-1
20 FTKLDRIGKGSFGEVYKGIDNHT HPK-2
81 QQINLGPSSNPHAKPSDFHFLKVIGKGSFGKVLLARHKAE HPK-3
2 FEMVDQKAR GI 1082115
30 FDVLEKLGEGSYGSVYKAIHKET GI 1117791
81 QQINLGPSSNPHAKPSDFHFLKVIGKGSFGKVLLARHKAE GI 294637
8 QDCVKEIGLLKQLNHPNII HPK-1
43 KEVVAIKIID---LEEAEDEIEDIQQEITVLSQCDSPYIT HPK-2
121 EVFYAVKVLQKKAILKKKEEKHIMSERNVLLKNVKHPFLV HPK-3
11 QDCLKEIDLLKQLNHVNVI GI 1082115
53 GQIVAIKQVP---V---ESDLQEIIKEISIMQQCDSPHVV GI 1117791
121 EAFYAVKVLQKKAILKKKEEKHIMSERNVLLKNVKHPFLV GI 294637

FIGURE 4A

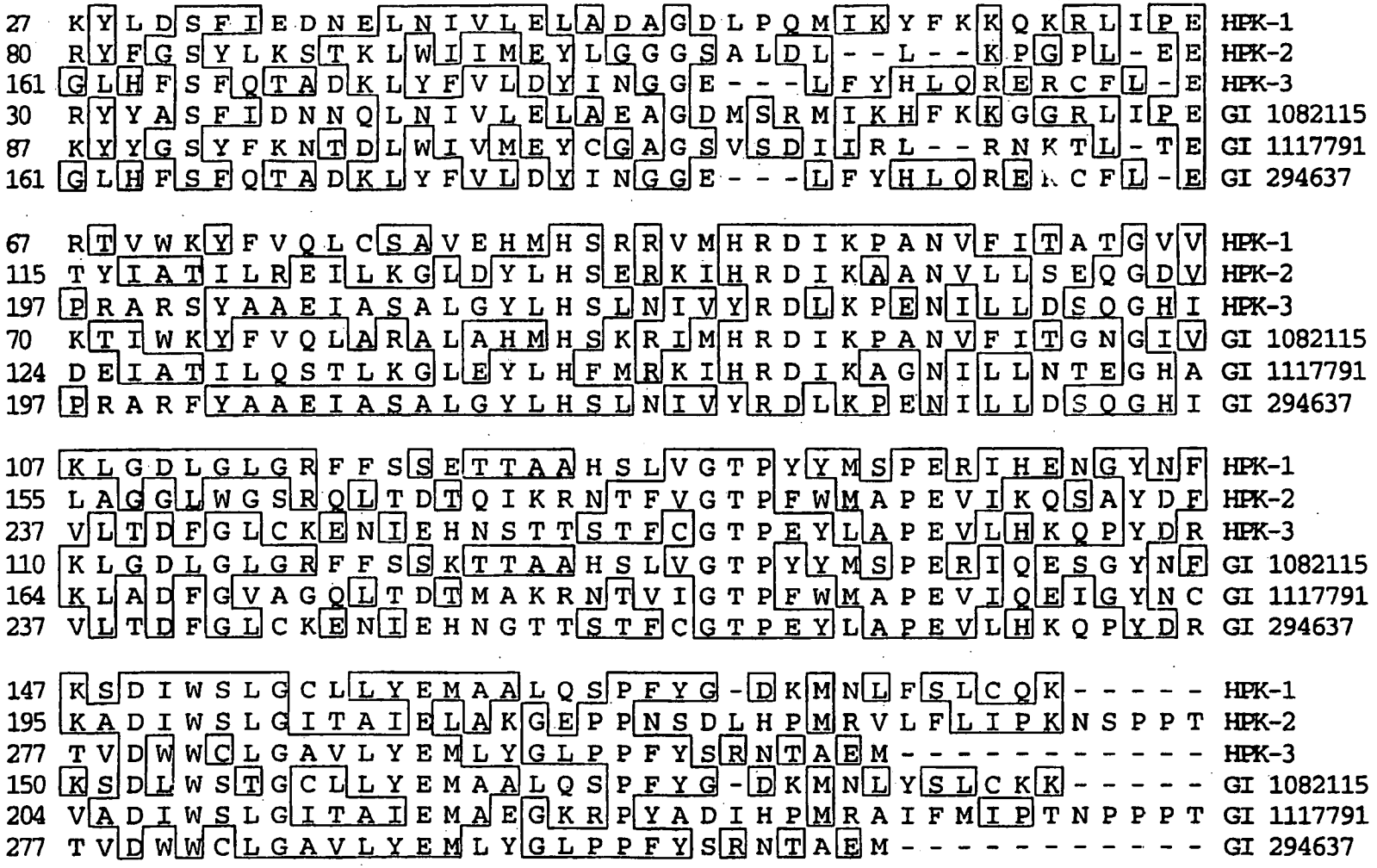
27 KYLDSFIEDNELNIVLELADAGDLPQMIKYFKKQKRLIPE HPK-1
80 RYFGSYLKSTKLWIIMEYLGGGSALDLL--L--KPGPL-EE HPK-2
161 GLHFSFQTADKLYFVLDYINGGE---LFYHLQREREFL-E HPK-3
30 RYYASFIDNNQLNIVLELAEAGDMSRMIKHFKKGGRLIPE GI 1082115
87 KYYGSYFKNTDLWIVMEYCGAGSVSDIIRL--RNKTL-TE GI 1117791
161 GLHFSFQTADKLYFVLDYINGGE---LFYHLQRE.CFL-E GI 294637
67 RTVWKYFVQLCSAVEHMHSRRVMHRDIKPANVFITATGVV HPK-1
115 TYIATILREILKGLDYLHSERKIHRDIKAANVLLSEQGDV HPK-2
197 PRARSYAAEIASALGYLHSLNIVYRDLKPENILLDSQGHI HPK-3
70 KTIWKYFVQLARALAHMHSKRIMHRDIKPANVFITGNGIV GI 1082115
124 DEIATILQSTLKGLEYLHFMRKIHRDIKAGNILLNTEGHA GI 1117791
197 PRARFYAAEIASALGYLHSLNIVYRDLKPENILLDSQGHI GI 294637
107 KLGDLGLGRFFSSETTAAHSLVGTPYYMSPERIHENGYNF HPK-1
155 LAGGLWGSRQLTDTQIKRNTFVGTPFWMAPEVIKQSAYDF HPK-2
237 VLTDFGLCKENIEHNSTTSTFCGTPEYLAPEVLHKQPYDR HPK-3
110 KLGDLGLGRFFSSKTTAAHSLVGTPYYMSPERIQESGYNF GI 1082115
164 KLADFGVAGQLTDTMAKRNTVIGTPFWMAPEVIQEIGYNC GI 1117791
237 VLTDFGLCKENIEHNGTTSTFCGTPEYLAPEVLHKQPYDR GI 294637
147 KSDIWSLGCLLYEMAALQSPFYG-DKMNLFSLCQK----- HPK-1
195 KADIWSLGITAIELAKGEPPNSDLHPMRVLFLIPKNSPPT HPK-2
277 TVDWWCLGAVLYEMLYGLPPFYSRNTAEM---------- HPK-3
150 KSDLWSTGCLLYEMAALQSPFYG-DKMNLYSLCKK----- GI 1082115
204 VADIWSLGITAIEMAEGKRPYADIHPMRAIFMIPTNPPPT GI 1117791
277 TVDWWCLGAVLYEMLYGLPPFYSRNTAEM---------- GI 294637

FIGURE 4B


181 - - - - - - - - - - - - - - - - I E Q C - HPK-1
235 L E G Q H - - S K P F K E F V E A C L N K D P R F R P T A K E L L K H K F I T R HPK-2
306 - - - - - - - - - - - Y D N I L N K P L Q L K P N I T N S A R H L L E - - - - - - - HPK-3
184 - - - - - - - - - - - - - - - - I E N C - GI 1082115
244 F R K P E L W S D N F T D F V K Q C L V K S P E Q R A T A T Q L L Q H P F V - R GI 1117791
306 - - - - - - - - - - - Y D N I L N K P L Q L K - N I T N S A R H L L E - - - - - - - GI 294637
185 - HPK-1
273 Y T K K T S F L T E L I D - - - - - R Y K R W K S E - - - - G H G E E S S S E D HPK-2
330 - - - - - - - - - - G L L Q K D R T K R L - HPK-3
188 - GI 1082115
283 S A K G V S I L R D L I N E A M D V K L K R Q E S Q Q R E M D Q D D E E N S E E GI 1117791
329 - - - - - - - - - - G L L Q K D R T K R L - GI 294637
185 - D Y P P L P G E H Y S E K L R - - - - - HPK-1
304 S D I D G E - - - - A E D G E Q G P I W T F P P T I R P S P H S K L H K G T A L HPK-2
341 - - - - - - - - - - - - - - - - - - G A K D D F M E I K S H V F F S L I N W D D L I HPK-3
188 - E Y P P L P A D I Y S T Q V S - - - - GI 1082115
323 D E M D S G T M V R A V G D E M G T V R V - A S T M T D G A N T M I E D D T L GI 1117791
340 - - - - - - - - - - - - - - - - - - G A K D D F M E I K S H I F F S L I N W D D L I GI 294637
200 - - E L V S M C I C P - - - - - - - - - - - - - - - - - D P H Q R P D - I G X V H HPK-1
340 H S - - - - - - - - - S Q K P A E P V K R Q P R S Q C L S T L V R P V F - - - - - HPK-2
365 N K K I T - P P F N - - - P HPK-3
203 - - - - A N L C F V Q - - - - - - - - - - - - - - - - L S S A T W Y P - V V Y F Q GI 1082115
362 P S Q L G T M V I N A E D E E E E G T M K R R D E T M Q P - A K P S F L E Y F E GI 1117791
364 N K K I T - P P F N - - - P GI 294637

FIGURE 4C

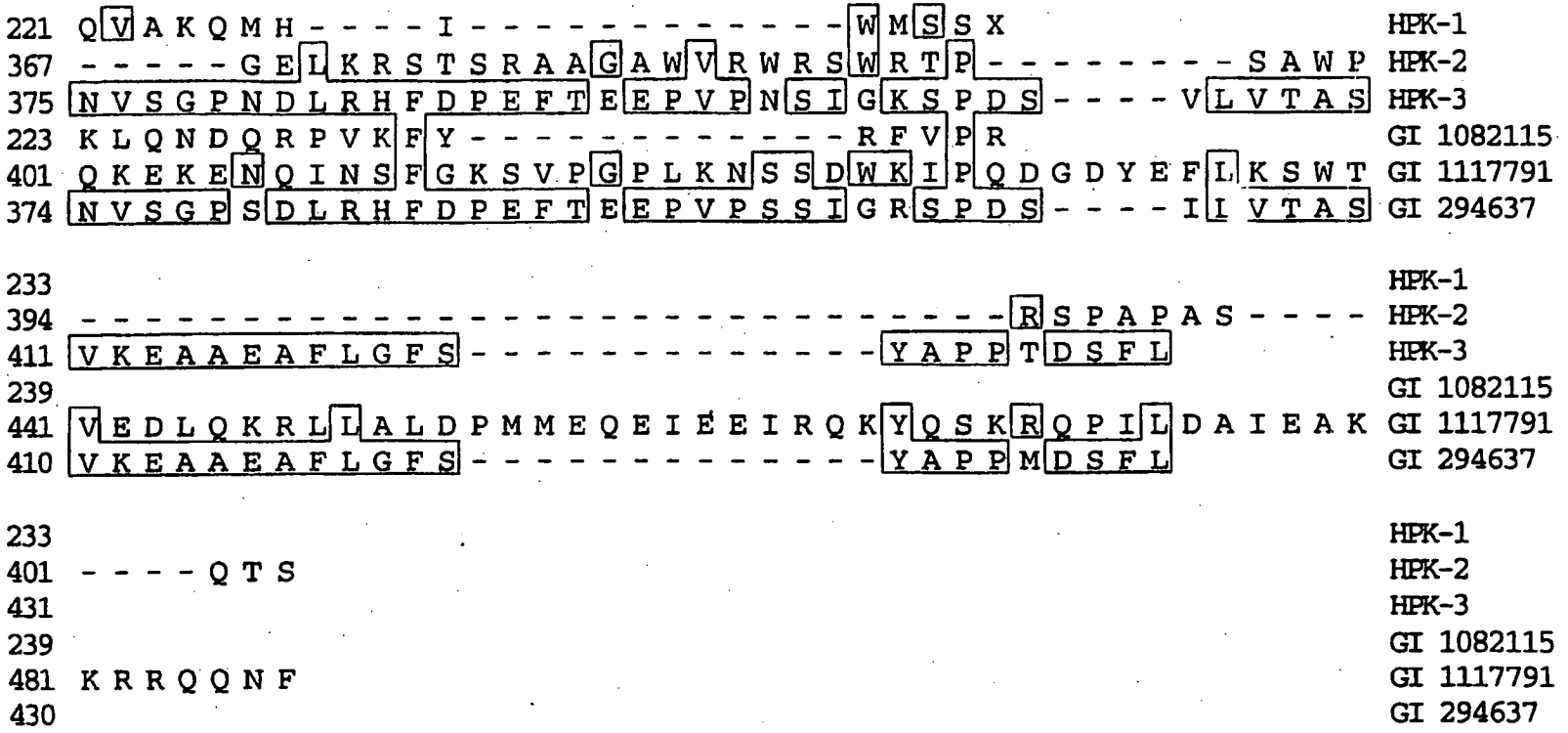
221 Q V A K Q M H - - - - I - - - - - - - - - - - - - W M S S X HPK-1
367 - - - - - - G E L K R S T S R A A G A W V R W R S W R T P - - - - - - - - - S A W P HPK-2
375 N V S G P N D L R H F D P E F T E E P V P N S I G K S P D S - - - - V L V T A S HPK-3
223 K L Q N D Q R P V K F Y - - - - - - - - - - - - - - R F V P R GI 1082115
401 Q K E K E N Q I N S F G K S V P G P L K N S S D W K I P Q D G D Y E F L K S W T GI 1117791
374 N V S G P S D L R H F D P E F T E E P V P S S I G R S P D S - - - - I I V T A S GI 294637
233 HPK-1
394 - R S P A P A S - - - - HPK-2
411 V K E A A E A F L G F S - - - - - - - - - - - - - - - Y A P P T D S F L HPK-3
239 GI 1082115
441 V E D L Q K R L L A L D P M M E Q E I E E I R Q K Y Q S K R Q P I L D A I E A K GI 1117791
410 V K E A A E A F L G F S - - - - - - - - - - - - - - - Y A P P M D S F L GI 294637
233 HPK-1
401 - - - - Q T S HPK-2
431 HPK-3
239 GI 1082115
481 K R R Q Q N F GI 1117791
430 GI 294637

FIGURE 4D